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DIFFERENCES BETWEEN ANTERIOR PITUITARY SEX-STIMULATING HORMONES AND PREGNANCY-URINE SUBSTANCES AS TESTED IN THE MALE MAMMAL AND BIRD[1]

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From the experiments with anterior pituitary extracts and pregnancy urine, especially in female rodents, many authors have inferred that the active substance from pregnancy urine is identical with the hypophyseal sex-stimulating hormone, particularly Zondek and Aschheim (1928).[3]

Some authors (Engle, 1929; Evans and Simpson, 1929; Orban and Watrin, 1929; Bourg, 1931) have questioned whether the two substances are identical inasmuch as prolan shows in female rats and mice an excessive luteinizing activity, whereas sex-stimulating pituitary extracts exert both the follicle-stimulating and luteinizing action in a normal physiological relation. Experiments of Evans and Simpson (1929), Collip and coworkers (1931) and Fluhmann (1932) have shown that there is a limitation to the increase in size of the immature female rat ovary induced in a given period with pregnancy-urine or -serum extracts but that such is not the case when pituitary extracts are used.

Evans, Meyer, Reichert and their collaborators (1932) claim that prolan is relatively ineffective in hypophysectomized rats and dogs, in contrast with pituitary extracts. Leonard (1932) found that there was a quantitative difference in the minimal ovulation dose for the rabbit between a pituitary extract and pregnancy urine extract. Also Engle (1932) demonstrated differences in the action of anterior pituitary substances and prolan

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[3] In the German literature, the term "Hypophysenvorderlappenhormon" (HVH) is applied indiscriminately to hypophyseal extracts and pregnancy urine, a usage which is not infrequent in this country also.

on the male Macacus rhesus. Yet Zondek (1932) still considers urinary prolan to be identical with the anterior pituitary sex-hormone.

Most of the experiments cited above were performed on female animals. The data presented in this paper will show that prolan can be differentiated from hypophyseal sex-hormones in the male rat and bird as well. In the male rat a quantitative difference in their respective action on the testes and the accessories was found; moreover, in ducks and fowl a sharp qualitative difference between sex-stimulating pituitary substance and pregnancy urine was established.

EXPERIMENTAL. As a criterion of action of the gonadotropic hormones in rats, the weight response of the testes and of the secondary sex organs, including the reproductive tracts, was used. In the birds the testes were weighed, the combs measured as a criterion of testicular endocrine stimulation, and the testicular changes histologically studied.

TREATMENTS. *Anterior pituitary preparations* have been administrated in various forms: *a*, a centrifuged saline suspension of fresh beef anterior pituitary tissue prepared as described previously, (Schockaert, 1931 b); the total amount given during the course of treatment varied from 0.5 to 3.0 grams of fresh anterior lobe tissue; *b*, a neutralized alkaline extract of dried, beef anterior-pituitary powder prepared according to the procedure of van Dyke and Wallen-Lawrence (1930); the total amount given to rats varied from 0.018 to 0.6 gram of powder. This extract, known as phyone, promoted growth in hypophysectomized rats, while in some experiments it had no effect on the ovary of immature female rats. Phyone from the Wilson Laboratories has also been used in a few animals.[4] *c*, a pyridine extract of dried beef anterior-lobe powder prepared according to the procedure of Fevold Hisaw and Leonard (1931): the two fractions, water-soluble and water-insoluble, have been injected separately in some animals and mixed in corresponding amounts in others.

Pregnancy urine of the 5th month was extracted with 95 per cent alcohol precipitation, and the oestrin was removed with ether in order to avoid any possible antagonistic effect on the gonad-stimulating action of the recipient's pituitary (Meyer, Leonard, Hisaw and Martin, 1930; Moore and Price, 1932). The total amount of extract given during the course of treatment was equivalent to from 24 to 120 cc. of pregnancy urine. It was tested in female immature rats. A commercial extract from pregnancy urine (Follutein, Squibb)[4] was also used.

EXPERIMENTS ON RATS.[5] The 102 male piebald rats used in the present

[4] We express our sincere thanks to Doctor Klein who supplied us with the Phyone (Wilson, Chicago), and to Doctor Morrell who furnished us the Follutein (Squibb, New Brunswick).

[5] The detailed experimental data, which were not published in order to conserve journal space, will be sent on request.

work ranged in age at autopsy from 28 to 65 days. The duration of the treatment varied from 5 to 22 days ranging in most of the cases from 7 to 11 days. No substantial difference in the response was observed with the length of treatment.

TABLE 1

The difference, irrespective of the dosage used, between the effect of pregnancy urine extract and pituitary extracts on weights of the testes and the accessories of the rat as shown by the genokinetic ratio (rats aged from 34 to 63 days at autopsy)

1. PREGNANCY URINE (5–9 DAYS)		2. ALKALINE EXTRACT OF DRIED ANTERIOR PITUITARY POWDER (8–9 DAYS)		3. SALINE SUSPENSION OF FRESH ANTERIOR PITUITARY (5–22 DAYS)	
Total amount injected	Genokinetic ratio N	Total amount*	Genokinetic ratio N	Total amount fresh tissue	Genokinetic ratio N
cc.		*grams*		*grams*	
2.4	1.91	0.018	1.40	0.5	1.41
2.4	1.56	0.018	1.57	0.8	0.51
5.5	2.53	0.090	1.12	0.9	1.34
7.0	2.19	0.090	1.53	0.9	1.56
7.0	2.19	0.090	1.12	0.9	1.42
10.0	3.09	0.180	1.28	0.9	1.63
10.0	2.86	0.180	1.50	1.0	1.06
10.0	1.71	0.360	0.84	3.0	1.48
10.0	1.83	0.450	1.50		
12.0	2.49	0.450	3.03 (?)		
18.0	2.53	0.800	1.01		
18.0	2.59	0.900	1.22		
18.0	3.17	0.900	1.31		
18.0	3.21	1.600	0.73		
24.0	2.58				
24.0	4.60				
35.0	2.55				
35.0	2.46				
60.0	2.17				
60.0	1.88				
64.0	2.09				
120.0	2.51				
120.0	2.80				
Average....	2.45		1.27		1.22

* In the tables the total amount of extracts injected is given in the corresponding quantity of dried beef pituitary powder which had been used for its preparation.

In preliminary experiments, the various preparations were given to different litters. However, in most of the later experiments, different preparations were given to animals in the same litter and the weights of their reproductive organs compared with that of the untreated littermate, as shown in table 2.

The percentage weights of the organs of the experimental animals were compared with those of the untreated control littermates which were considered as unity, inasmuch as control littermates show only small differences between the percentage weights of their organs, this procedure of quantitative comparison seems justified.

Effects of the injections on the testes. Prolan and the pyridine extracts of dried beef anterior lobe powder have induced in young immature rats an

TABLE 2

Comparative effect of pituitary extracts and pregnancy urine extracts on testes and accessories in young immature littermate rats, less than 34 days old. Shows type of protocol used in calculating the genokinetic ratio

NUMBER OF ANIMALS	TREATMENT			WEIGHT OF ANIMAL	WEIGHT OF TESTES			WEIGHT OF SECONDARY ORGANS			GENOKINETIC RATIO N
	Daily injections				ABSOLUTE	Per 100 grams body weight	Relative per cent, weight control being 1.00	Absolute	Per 100 grams body weight	Relative per cent, weight control, 1:00	
33 days old	Substance	Amount*	Number of days								
				grams	*grams*			*grams*			
BH6725	None		0	45	0.145	0.323	1.00	0.153	0.340	1.00	1.00
GH6728	Prolan	45 cc.	9	50	0.486	0.972	3.00	0.579	1.059	3.10	1.03
GH6727	Pyridine WS†	2.0 gm.	9	57	0.385	0.676	2.09	0.189	0.331	0.97	0.46
GH6726	Pyridine total	2.2 gm.	9	57	0.425	0.746	2.30	0.267	0.468	1.37	0.59
GH6729	Pyridine WI	2.4 gm.	9	64	0.627	0.980	3.03	0.275	0.429	1.26	0.41
28 days old											
GH6774	None		0	40	0.146	0.366	1.00	0.155	0.387	1.00	1.00
GH6777	Prolan	40 cc.	8	41	0.344	0.840	2.29	0.538	1.312	3.39	1.48
GH6775	Alkaline extract	1.6 gm.	8	35	0.258	0.591	1.61	0.157	0.448	1.15	0.71
GH6776	Alkaline extract	1.6 gm.	8	40	0.315	0.789	2.15	0.247	0.618	1.59	0.73

* See note on table 1.

† WS: water soluble fraction; WI: water insoluble fraction.

increase of weight of the testicles up to 203 per cent as compared to the control animals. It appears, however, that very marked effects are observed only in relatively young animals (table 2). As the rats grow older and their testes approach more and more the adult size, no increase in weight usually obtains even with increased dosages.

In the rats over 35 days of age, indeed, as already reported by others, the potent extracts from the pituitary or from pregnancy urine induced in some cases not an increase but a loss in weight, even when the dosage was small and a toxic effect improbable.

Within the range of dosage used in the present experiments there is apparently no direct relation between the dosage administered and the weight response of the testicles among animals of the same age group, in contrast to the female, where the ovarian weight responses may be used as a relatively safe quantitative index.

As a whole, the 14 animals treated with the alkaline extract gave the same average testicular weight as the untreated controls, the 8 treated with fresh suspension showed an increase of 24 per cent, and the 22 treated with prolan showed a loss of weight of 16 per cent. There was a rather wide variation in the response, however, especially in the rats injected with prolan and the alkaline pituitary extract.

Response of the secondary organs. Both pituitary and urinary substances induced an increase in weight of the accessories reaching in a single instance 249 per cent after a pregnancy urine treatment.

Although here also younger animals usually showed a greater response than the older ones to a given amount, as already noted by Collip et al. (1931) and Moore and Price (1931), no definite relation in animals of a given age group obtained between the dosage used and the response, no matter whether the injected substance was prepared from pituitary or pregnancy urine.

EXPERIMENTS ON BIRDS. The writer has previously shown (1931a, b) that the testes of the immature duck constitute a sensitive test for the gonad-stimulating hormone of the pituitary (gametogenic function). Moreover, comb growth being a sensitive and specific test for the male hormone (McGee, Juhn and Domm, 1928; Freud, de Frémery and Laqueur, 1932), the immature chick seemed particularly adapted for a comparative study of sex-stimulating substances on the endocrine as well as on the gametogenic functions of the testes.

Table 3 embodies data on 19 male immature brown Leghorns from the same hatching, and aged 34 days at the beginning of the treatment which lasted 17 days. They were in good general condition, an important factor as far as comb-reaction is concerned, as shown by Freud and co-workers (1932).

Effect on the testes. Allowing for the individual variations which are greater in birds than in mammals, it is certain that pituitary substances, pyridine as well as alkaline extracts, possess a marked stimulating action on the growth of the testes of immature birds. Histological study shows that the increase in weight seems due only to the increase in the size and cellular content of the tubules, the interstitial cell-masses showing no increase.

The fowls showed a stimulation of the spermatogenic activity similar to that observed in ducks (Schockaert, 1931a, b) treated with fresh pituitary or phyone. While in the control chicks only one or two layers of

primordial germ cells were present, the testes of the treated animals, especially with the water soluble fraction of pyridine extracts, show maturing tubules. Free spermatozoids were observed several weeks before the time of spontaneous onset of spermatogenesis, showing conclusively the stimulating action of pituitary on spermatogenesis.

In contrast with this, pregnancy urine extracts, even at the relatively high dosage of 80 cc., failed to induce any increase in weight of the testes or any stimulation of the tubules. In fact, the diameter of many tubules had materially decreased showing either a toxic or an antagonistic effect, as illustrated also by a decrease in weight or at least a failure in growth of the testes. In the duck, Follutein (Squibb) and prolan, which are both

TABLE 3

Comparative effects of various pituitary extracts and of pregnancy urine extract on the testis-weight and the comb-growth in immature male chicks. Duration of experiment 17 days

NUMBER OF BIRDS	TREATMENT		AVERAGE WEIGHT	AVERAGE WEIGHT OF TESTES		AVERAGE SURFACE OF COMB			
	Substance	Total amount*		Absolute	Per 1000 grams	Before treatment	At kill	Increase	
								Absolute	Per cent
		grams	*grams*	*grams*	*grams*	*cm.²*	*cm.²*		
3	Controls		585	0.240	0.408	3.3	5.5	2.2	69
3	Alkaline extract	2.62	630	0.342	0.535	4.0	8.3	4.3	133
4	Pyridine extract (water soluble)	1.60	625	0.611	1.383	2.7	8.4	5.7	205
4	Pyridine extract (water soluble)	1.57	575	0.298	0.522	3.8	9.3	5.3	130
3	Pyridine extract (both fractions)	1.43	545	0.581	1.071	2.4	8.5	6.1	271
2	Pregnancy urine	67.5 cc.	460	0.116	0.206	2.5	3.8	1.3	54

* See note on table 1.

pregnancy urine extracts, have also been found ineffective on the immature testicle, histologically as well as macroscopically.

Effect on the comb. The comb measurements were taken according to de Frémery's (1930) method, the outlines being taken from actual silhouette photographs. The light was always at the same distance of two meters from the comb, and the latter applied directly against the sheet of sensitive paper. The comb areas were then measured with a planimeter and are given in square centimeters.

Measurements of the comb taken three times a week ruled out any temporary irregularity in the rate of growth which might have influenced the final measurement.

The pyridine and alkaline extracts from dried beef pituitary powder proved active in stimulating comb growth, as shown in table 3 and figure 1. This is due to stimulation of the endocrine function of the testes, since in two capons no stimulation of the atrophied comb with the alkaline pituitary extract was obtained.

In contrast to the pituitary substance, pregnancy urine extracts did not induce in the testes the production of male hormone as shown by their failure to stimulate comb growth.

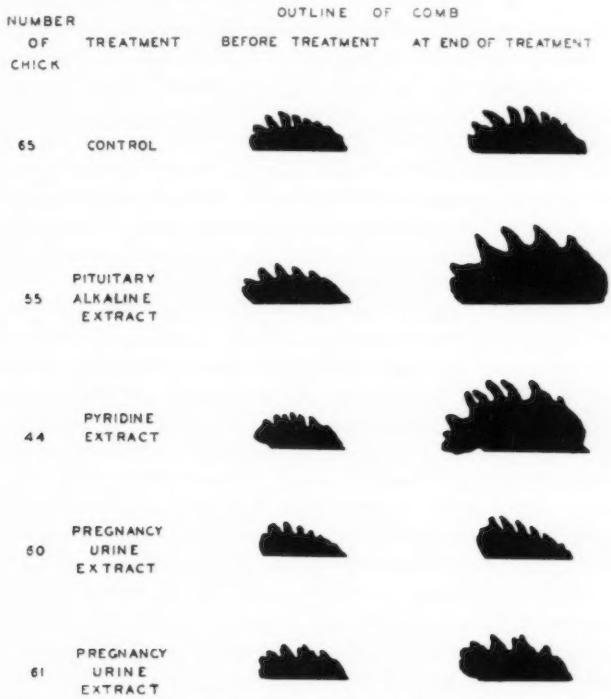

Fig. 1. Showing comparative effects of pituitary extracts and pregnancy urine extract on comb-growth in the immature male chick.

In conclusion, while pituitary extracts stimulate in the bird's testes both the gametogenic and the endocrine function, pregnancy urine fails to affect either one. The marked response to beef pituitary extracts and suspensions in our experiments in ducks and chicks, confirming Domm's (1931) results with homeo-implants in chicks and Riddle's (1928, 1931) observations with extracts and implants in doves and pigeons, proves that the inactivity of pregnancy urine is not due to a lack of sensitivity of the

bird testes. These experiments on birds thus sharply differentiate both substances.

Although pituitary hormones so markedly stimulate the internal secretion of male hormone as shown by the comb reaction, they failed to induce any appreciable hypertrophy of the interstitial cell masses as determined by the ordinary fixation and staining methods. The bird testes definitely differ from the mammalian in this respect.

Discussion. The variation in the weight responses of the testes and accessories of the rat after both prolan and pituitary treatment at first sight apparently failed to demonstrate a difference in the action of these two substances in the male, as was previously admitted. A more thorough analysis of the respective quantitative responses of testes and accessories, however, revealed a constant definite difference between the action of both substances. Dividing the relative change in weight of the accessories as compared to the untreated control taken as unity, by the corresponding testis figure, a testes-accessories ratio was obtained, which may be called ratio of sex-system stimulation, or "genokinetic ratio,"[6] designated in the tables by N. By definition, this ratio is 1.00 for the control littermate. (See table 2.)

Within a group of rats of the same age, whether littermates or not, the range of variation of the ratio is practically constant for animals treated with the same substance and it is independent of the dosage given.

The ratio, however, is distinctly and constantly different in the prolan-treated group and the pituitary-treated group. Indeed, animals above 34 days (table 1) fall into two classes, a pituitary group in which the ratio varies from 0.51 to 1.63 (with one exception to W7076) and a pregnancy urine group, in which the ratio ranges from 1.56 to 4.60, the averages being 1.27 and 1.22 respectively for the alkaline extract and the fresh pituitary suspension groups, and 2.45 for the pregnancy urine animals, the latter being nearly twice as high as the former. In rats below 34 days of age (table 2) the ratio, although smaller than in older animals owing to the increased growth of the testes, is also twice as high, however, for the pregnancy urine treated rats as for the pituitary treated animals.

Further analysis shows that this difference in the genokinetic ratio in the maturing rat is due only to the activity of the two substances.

1. Since the pregnancy urine extracts induced often a loss in the weight of the testes, due probably to some toxic nonspecific substance, a given increase of the accessories would seem relatively higher in the pregnancy urine group than in the pituitary group, and explains the difference in genokinetic ratio.

This is not the case, however, for notwithstanding the loss of weight of the testes in the pregnancy urine group, the relative and even absolute

[6] From the Greek: genos (sex) and kinō (to stimulate).

increase in weight of the accessories is still higher, in individual cases as well as in the group as a whole, the average relative percentage weight of the accessories being 2.07 for the pregnancy urine group as against 1.27 and 1.52 for the pituitary groups. Whenever in the latter groups the testes showed a loss of weight, the ratio remained low because of the small increase of the accessories, and inversely when in a pregnancy urine animal the testes had increased in weight the ratio went up also, because of the still greater increase in the weight of its accessories.

2. The objection that a difference in dosage might be responsible for the different genokinetic ratio is not justified, for in the present experiments a wide range of dosages was used. The extreme dosages varied indeed from 1 to 50 for the pregnancy urine, and from 1 to 88 for the alkaline pituitary extract without influencing the genokinetic ratio in either group.

Amounts of pregnancy urine extract equivalent to 2.4 to 7 cc. of urine gave the same index as did 60 and 120 cc., and in the alkaline extract series total amounts corresponding to 0.018 gram of dried powder gave the same index as 0.900 gram, or 50 times more.

3. It was shown (Schockaert 1931a, 1932) that pituitary emulsions and alkaline extracts in addition to the gonad-stimulating hormone, contain also a thyroid-activating hormone which was not found in pregnancy extracts. Hence the question arose as to whether or not a thyroid stimulation in the pituitary series might influence the sex response. This seemed not improbable, for it had been previously observed (1931c) that thyroidectomy prior to fresh pituitary suspension injections, was followed by a better response of the accessories, giving a higher genokinetic ratio than in the non-operated treated littermates. The reverse experiment, however, disproved this hypothesis, since in four litters, 0.30 to 0.40 mgm. of thyroxin (Roche) administered along with the pregnancy urine, did not bring down the genokinetic ratio as compared to the ratio of littermates treated with the pregnancy urine alone.

4. Finally, one may consider both substances as being a mixture of two different principles, possibly antagonistic, one of which would stimulate the endocrine activity of the testes, while the other would stimulate only their gametogenetic activity.

Were both principles present in a different concentration in pregnancy urine and pituitary extract, a discrepancy between the relative response of the testes and the secondary organs as revealed by the genokinetic ratio, would ensue. There is some evidence that supports this hypothesis: only with pituitary substance has full spermatogenesis been secured in immature birds (Schockaert, 1931b) and reported in mammals (Voss and Loewe, 1928; Belawenetz, 1930) while pregnancy urine even with high dosages never induces more than a mild stimulation of spermatogonial and spermatocytal divisions (Engle, 1929, 1932; Borst, 1930; Borst and

Gostimirović, 1930; Kraus, 1930, 1931; De Jongh, 1930; Boeters, 1931; Bourg, 1931; Neumann, 1931; Moore and Price, 1931). Inversely we have been able to obtain in rats with pituitary substances only occasionally a slight increase of the interstitial masses, which is in agreement with findings on the effect of implants (Smith and Engle, 1927) while pregnancy urine, as well known, always induced a marked hypertrophy of the interstitial tissue.

This explanation, while satisfactory for rats, does not, however, explain the facts observed in birds. Indeed even at relatively small dosages, fresh pituitary suspensions and alkaline and pyridine extracts of dried pituitary powder stimulated in immature birds both the gametogenic and endocrine functions of the testes,[7] while the pregnancy urine extracts fail to stimulate either function. Assuming that the pituitary substance which stimulates the male gonad is the same as that which stimulates the female gonad, as is usually accepted, these results show that pituitary and pregnancy urine substances can no longer be considered as identical substances. It is strongly suggested that the term *anterior pituitary* extracts should be applied exclusively to those made from the hypophyseal tissue and not to those made from pregnancy urine.

SUMMARY AND CONCLUSIONS

A comparative study of the effect of anterior pituitary substances and of pregnancy urine extract on the male genital system was made in the immature mammal (rat) and bird (duck, chick).

In the rat, both pituitary and pregnancy urine extracts induced an increase in weight of the testes in animals below 35 days of age. In older animals, both may induce a loss as well as a gain in weight, but always induce an increase in weight of the accessories. Although the results fail to show in rats a qualitative difference between both groups of substances, they demonstrate, however, a quantitative difference shown by a different ratio of sex-stimulation, or genokinetic ratio, which is obtained by dividing the relative change in weight of the accessories by that of the testes, as compared to the control littermates. In the same age groups, the genokinetic ratio varies within a rather small range, and is independent of dosage. It was twice as high for the pregnancy urine treated rats as for the corresponding pituitary treated animals.

[7] The fact that the penis in the duck responds very slowly to the endocrine secretion of the testes (Champy, 1931) explains why in a previous paper a possible dissociation between the gametogenetic and endocrine functions of the testes in their response to pituitary was considered. (Schockaert, 1931b.) Hence the failure of the penis to grow after a pituitary treatment which markedly stimulated testicular growth, was not necessarily due to an absence of stimulation of testicular endocrine activity. The present experiments on the chick definitely show that both testicular functions are stimulated by pituitary hormones.

In the immature duck and fowl the beef pituitary substances induced a marked increase in weight of the testes. Full spermatogenesis was induced long before the time of spontaneous testicular maturity, but without noticeable hypertrophy of the intertubular tissue. In the chick, an increased rate of growth of the comb was constantly obtained after pyridine and alkaline extracts.

Pregnancy urine extracts, however, proved inactive in these forms both as regards the endocrine and the gametogenic function, no increased growth of the testes or the comb being observed.

From the data presented, it seems safe to conclude that although prolan and pituitary extracts show in the male mammal some similar properties, they appear to be distinctly different substances.

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[8] For references not given above, the reader is referred to the bibliographies of articles by Engle, Moore and Smith in *Sex and Internal Secretion*, Allen.

ELECTRIC RESPONSES IN THE SUBMAXILLARY GLAND

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There are good reasons to believe that the excitatory process in the submaxillary gland (Babkin, Stavraky and Alley, 1932) is similar to that which probably takes place in smooth muscle; i.e., the nerve impulse sets free a given amount of an acetylcholine-like (parasympathetic) or an adrenin-like (sympathetic) substance M, M combines with an excitatory or inhibitory substance H, and the response is proportional to the amount of MH produced (see Rosenblueth, 1932). The analysis of the electrical potentials developed in smooth muscle innervated by sympathetic nerves revealed several phenomena consistent with the hypothesis mentioned above for the excitatory process (Rosenblueth, Leese and Lambert, 1933). It was therefore deemed desirable to study the electrical potentials in the submaxillary gland, and attempt to correlate them with those obtained from smooth muscle.

Since Bayliss and Bradford (1885) first reported the electrical phenomena which attend activity of the salivary glands, several studies have appeared on the subject (see Harreveld, 1930, for a summary of the literature). Unfortunately this material was, however, hardly useful for the purpose at hand, for the following reasons: very few records have been presented, almost all of them being schematic drawings of the excursions of different types of galvanometers; no amplification was used; the electrodes leading from the glands to the galvanometers were diffuse, collecting the composite changes of potential from many cells; finally, only a few authors deal with the results of single shocks applied to the nerves.

METHOD. Cats were used, under dial anesthesia. The chorda tympani and the cervical sympathetic were stimulated by means of induction shocks (usually maximal) from a balanced inductorium (Bishop, Erlanger and Gasser, 1926), through shielded wire electrodes applied to the cut chordo-lingual nerve and to the sympathetic chain in the neck. Tetanizing frequencies were obtained from a Harvard inductorium. Injections of pilocarpine, atropine and cocaine were given into the femoral vein.

The string galvanometer and the recording camera were those described by Forbes, Davis and Lambert (1930). The amplifiers used were the condenser-coupled unit employed by Rosenblueth, Leese and Lambert (*loc.*

cit.) and a new direct-coupled unit (Garceau and Forbes, 1933), which was designed expressly for the purpose of recording slow responses without distortion. Two strings were used in the galvanometer, referred to as nos. 1 and 2. The first had a resistance of 1,000 ω, and the second 5,000 ω.

In the earlier experiments the string in the galvanometer was fairly tight. In the later experiments—almost all those with the direct-coupled amplifier—a very slack string was employed. The reason for this was that the amplifier magnifies all oscillations, rapid and slow alike. The electric responses in the gland are much slower than the fortuitous oscillations in the base line. Slacking the string magnifies slow excursions much more than rapid ones; therefore, by using only a moderate degree of electron tube amplification and a slack string, the excursions we sought to record were magnified without corresponding amplification of the undesirable irregularities of the base line. The tensions employed were from 6 to 12 meters per ampere in the earlier experiments, and from 200 to 300 meters per ampere in the later ones. This is a measure of the absolute tension of the string in meters of actual motion per ampere traversing it, which means in practice microns per microampere. The absolute tension in these units is derived from the observed tension in millimeters per millivolt applied to the string by the following formula: $T = \frac{DS}{M}$, where T is the tension expressed in meters per ampere, D, the deflection of the magnified shadow in millimeters per millivolt, S, the resistance of the string in ohms, and M, the magnification (cf. Forbes and Ray, 1923).

The leads to the amplifier were concentric needle electrodes (Adrian and Bronk, 1929), the diameter of the copper core was 0.3 mm., and that of the steel needle 0.6 mm. The core was connected to the grid and the needle to the ground.

RESULTS. A. *Single shocks on the chorda tympani.* A complex electric response is obtained. For reasons which will be given in the discussion we shall divide this response into two parts, a "quick" and a "slow" component; the latter is frequently complex. Figure 1 illustrates a complete response, with its two parts. It is rather seldom, however, that the two effects appear as clearly in a single observation. It is common to obtain at first only the quick component; then, as the stimulus is repeated, the quick effect progressively declines, possibly because of accumulation of saliva around the electrodes, while the slow one increases, finally to become the only response obtained. Or again, only one of the components may appear with certain positions of the leading-off electrodes, difficult to determine.

The condenser-coupled amplifier used in early experiments made the quick response appear diphasic, as shown in figure 2A. This, however, is merely a distortion dependent on the rapid discharge of the condenser in

the amplifier. The direct-coupled amplifier, which gives an undistorted picture of the electrical disturbance, shows almost invariably a fairly pure monophasic record (fig. 2B), although in a few instances, particularly on repetitive stimulation (two shocks), a diphasic character was distinct (fig. 4F). The direction of the monophasic records showed invariably electronegativity of the localizing core with respect to the diffuse ground lead.

Fig. 1. Responses to a single shock. Q = quick effect; S = first phase of the slow component. μv/mm = 10.0. In this and other figures, the stimulus artefact is indicated by the sharp spike in the string record, or by arrows; the time is marked in 40 σ intervals. The amplification is given in μv/mm of excursion in the figures.

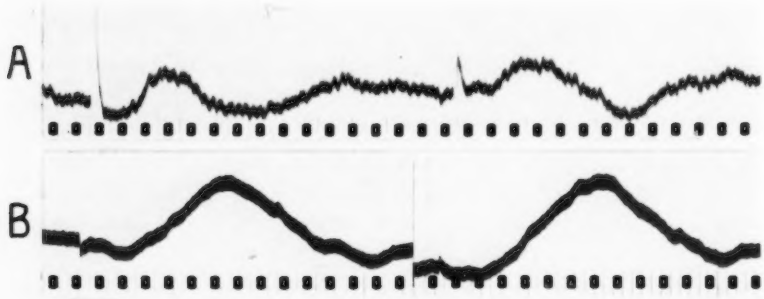

Fig. 2A. Distorted quick responses appearing as diphasic with a condenser-coupled amplifier. μv/mm = 2.7.

B. Monophasic quick responses to make and break stimuli recorded with a direct-coupled amplifier. μv/mm = 9.6.

The latency of the quick effect is usually about 250 to 300 σ. It is variable in the same preparation and with a given position of the electrodes. As a rule, repetitive stimulation shortens the latency. The duration of the deflection is likewise variable, from 500 to 800 σ. In general, big excursions last longer than smaller ones.

The slow component is longer (15 seconds to 1 minute). It presents wide differences in shape, duration and direction, from one preparation to another. It varies, also, with different positions of the electrodes. These differences may be interpreted on the basis of further subdivision

into two waves. In the first, the grid is negative, in the second, positive with respect to the ground lead. The first wave is shorter than the second one. Either of the two may be absent. Figure 3 illustrates this description. Record A is an example of the first wave only of a slow effect; the quick component is absent. Record B illustrates the first wave of the slow effect and the beginning of the second wave; the string returned to its original position about 30 seconds later.

B. *Repetitive stimulation of the chorda tympani.* The results of repetitive stimulation of the chorda tympani may be readily explained by assuming an independent summation of both the quick and the slow components.

As stated above, single shocks repeated at relatively long intervals (30 seconds to 1 minute) usually elicit quick responses of decreasing magnitude, which may finally disappear.

Two maximal shocks delivered at shorter intervals (below about 2 seconds) give pictures of partial summation of the quick effect (fig.

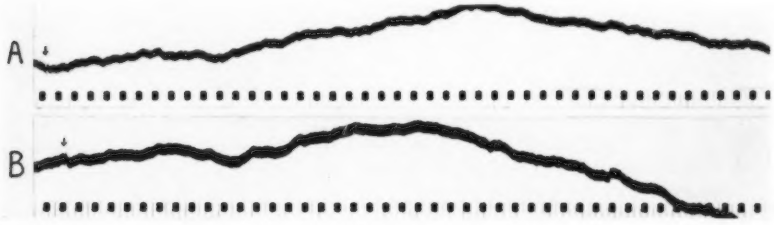

Fig. 3A. First phase of slow effect with no quick response. μv/mm = 17.8.
B. Similar response showing in addition, the beginning of the second phase of the slow component which lasted for several seconds. μv/mm = 15.1.

4A, B and C) until finally the two deflections fuse into a single large excursion (fig. 4D). This summation is particularly striking when the response to the first shock is smaller than usual, as in a fatigued preparation (fig. 4E and F).

Similar remarks apply to the slow component, but the summation interval is much longer.

When several shocks are administered in succession, if the interval is not too short, a series of summated quick waves may appear (fig. 5A). If the intervals are shorter, as, for instance, with tetanizing frequencies, there is an initial large summated deflection, a succeeding state of electrical equilibrium and finally the slow effect (fig. 5B).

As regards the slow component on a series of stimuli, the response appears after stimulation ceases, and is bigger and longer, the greater the number of stimuli applied. Tetanic stimulation may evoke a series of rhythmic waves instead of the usual slow effect (see section F and fig. 7D).

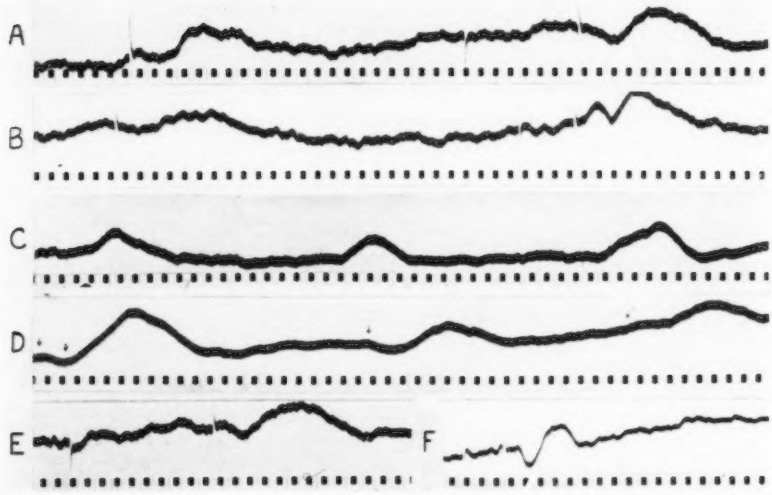

Fig. 4. Single and summated responses to paired maximal stimuli, at decreasing intervals. In all of these, single make and break shocks gave equally small responses.

A and B. Partially summated responses. μv/mm = 11.4.

C. Same. μv/mm = 32.9.

D. Complete summation. Note that the summated response precedes the smaller single responses. μv/mm = 28.9.

E. An increased response to a second stimulus after fatigue of the response to a single shock. μv/mm = 11.0.

F. Same but diphasic, an effect occasionally obtained with a pair of stimuli. μv/mm = 17.8.

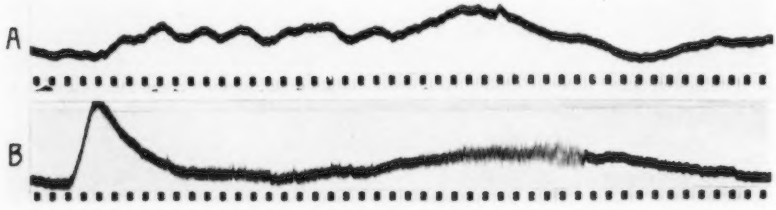

Fig. 5A. Summated quick responses to a series of single shocks at decreasing intervals. μv/mm = 30.9.

B. Response to tetanic stimulation showing large summated quick effect and first phase of slow effect. μv/mm = 45.7.

C. *Single shocks on the sympathetic.* In no instance did there appear any clean-cut electric response to a single shock applied to the cervical sympathetic. In some cases cocaine was previously injected, which (Rosenblueth, unpublished) increases the secretion obtained with a given stimulus, but it did not succeed in producing the appearance of responses. A few records show suspicious tracings occurring at consistent intervals

Fig. 6. Response to tetanic stimulation of the cervical sympathetic. Time indicated on record. μv/mm = 13.4.

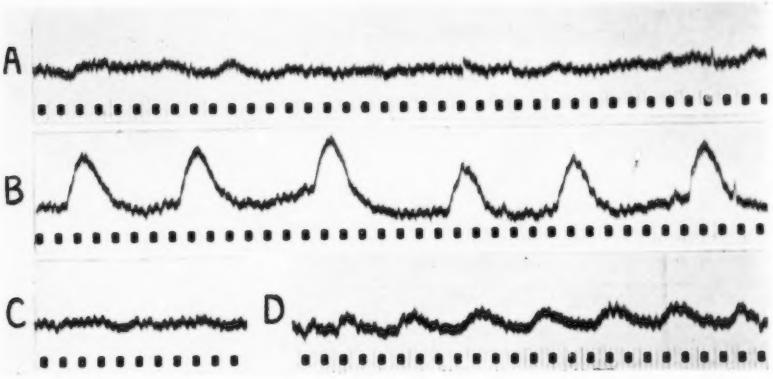

Fig. 7. Rhythmic responses obtained after injection of pilocarpine and tetanic stimulation. In all of these, μv/mm = 3.3.
A. Base line before injection.
B. Responses after injection of pilocarpine.
C. Base line immediately after tetanic stimulation.
D. Responses several seconds later.

after the stimuli; they are not sufficiently marked, however, to justify any conclusions.

D. *Repetitive stimulation of the sympathetic.* Tetanic stimulation of the cervical sympathetic gives responses quite similar to, though smaller than, those obtained from the same stimulation of the chorda tympani. Figure 6 illustrates typical results. By comparing this record with that obtained from chorda stimulation (fig. 5B) one may conclude that there are here, likewise, two components, a quick and a slow.

E. *Effects of ligating Wharton's duct.* The responses persist, apparently unchanged, for a long time (one hour or more) after the ligation (cf. Bradford, 1887).

F. *Pilocarpine.* An injection of pilocarpine gave rise in one instance to a rhythmic series of waves which started with the secretion of saliva and lasted throughout this secretion (fig. 7B). The same preparation, before pilocarpine, presented repeatedly similar rhythmic oscillations as a delayed response to tetanic stimulation of the chorda tympani (fig. 7D). This rhythmic effect was only doubtful in other preparations, and totally absent in some. The effect of pilocarpine in these latter cases was a prolonged, steady deflection such as had been previously described (Cannon and Cattell, 1916).

If the chorda tympani is stimulated while the gland is under the influence of pilocarpine, no electric responses ensue; the gland is apparently refractory. If the dose is small (e.g., 0.2 mgm.), excitability through the nerves reappears when the response to pilocarpine is over. Larger doses (e.g., several mgm.) seem to cause lasting injury to the gland, for even after the secretion of saliva has ceased, stimulation of the chorda does not give rise to any electric responses for several hours.

G. *Atropine.* Moderate doses (0.25 to 1 mgm. per kgm.), sufficient to paralyze secretion, abolish the quick phase of the electric response to chorda stimulation, both to single shocks and to tetani, leaving the slow component present, although reduced; larger doses abolish all electrical phenomena (cf. Bradford, 1887).

Discussion. Previous reports of the electrical potentials developed in the submaxillary gland during activity are complex and often contradictory. Thus Harreveld (1930) describes 15 different types of chorda-electrograms, not to mention additional types depending on the frequency of stimulation, repeated stimulation, etc.; Gayda (1924) speaks of 6 distinct phases in his records; Bradford (1888), Berk and Zbyszewski (1912) and Harreveld (1930) report an initial negativity of the surface of the gland with respect to the hilus on stimulation of the chorda with a single shock, while Rabl (1922) and Gayda (1924) find the surface to be first positive.

It is not surprising that the electrogram should be complex, since there are the following possible sources of potentials during activity of the gland: an excitatory wave, chemical and physical changes in the secretory cells, and movement of fluids (blood, saliva). The inconsistencies in the responses may be due to several factors; different frequencies of repetitive stimulation may, for instance, emphasize one of the sources of potential mentioned above; the same may be true for different intensities or durations of the stimuli. The probable influence of these factors on the electrogram was not studied in the present observations.

The type and position of the leading-off electrodes must likewise in-

fluence the electric response obtained. All previous observers have used diffuse leads applied to different portions of the surface of the gland or to the gland and some inactive neighboring structure. By use of localizing leads inserted into the gland we hoped to obtain a consistent electrogram. That we at least partly succeeded is shown by the description of our results and by the records presented, particularly as regards what we have called the quick effect.

The differences of potential obtained may be explained as occurring between an active localized region of the gland in contact with the core, recording against a diffuse statistical cancellation of the potentials in the elements in contact with the larger ground lead. They might also represent a resultant of the polarization of the gland, the deep core being nearer the hilus and the ground needle approximating the potential at the surface of the gland. The histological structure of the organ makes the first explanation more probable.

The interpretation of the electrograms, i.e., the particular events in the gland to which they are correlated, can only be tentative with the data available. As pointed out by Bradford (1887), they are not of muscular origin, because of the effects of atropine. They are also probably not produced by movement of fluids in the gland (Bradford, 1887).

Suitable doses of atropine (section G) permit the separation of the two components we have named quick (figs. 1 and 2) and slow (figs. 1 and 3). By analogy with the smooth muscle electromyogram (Rosenblueth, Leese and Lambert, 1933) one would be tempted to correlate the quick effect in the gland with the excitatory initial spike of the muscle. This assumption, however, is improbable for the following reasons: the long latency and longer duration (see section A); the summation on repetitive stimulation (see section B and fig. 4); the ready abolition by atropine. The long latency and duration are only quantitative differences. The phenomenon of summation, however, is a qualitative argument of importance. This summation is not analogous to that occurring in nerve (Levin, 1927; Amberson and Downing, 1929), which involves only the persistent, delayed potentials, and not the spike, for the quick potential of the gland, if excitatory, would be precisely the equivalent of the spike potentials.

It is well established that the transmission of the parasympathetic nerve impulse to the secretory cell is mediated by the production of an acetylcholine-like substance (Babkin, Stavraky and Alley, 1932). Since atropine does not abolish the production of the mediator in the case of heart muscle (Loewi, 1922), and since in the case of smooth muscle the excitatory initial potential is probably correlated with the production of the mediator (Rosenblueth, Leese and Lambert, *loc. cit.*), we may infer by analogy that if the quick component of the salivary electrogram were an initial excitatory potential of the same sort, it would probably not be abolished by atropine.

Furthermore, with an adequate dose of atropine not only is the quick potential suppressed, but the slow effect persists. If, then, the quick potential were excitatory and the slow effect were a response potential, we would have in these cases a response occurring without a stimulus. There remains, however, the possibility that the quick potential is not entirely abolished by atropine, but only attenuated till it becomes indistinguishable from the irregularities in the base line; this is unlikely in view of the previous arguments.

We are thus led to infer that the salivary electrogram is probably correlated exclusively with the secretory process. This does not imply that the other possible sources of potentials mentioned—excitatory process, contraction and relaxation of smooth muscle, movement of fluids—actually do not produce electrical disturbances in the gland. In all probability they do, but we failed to record them, as previous observers have similarly failed. We could only use a small amplification, for when it was increased the base line became too uneven for the differentiation of smaller effects.

The two components of the electric response being associated with the secretory processes, and one of the effects appearing alone after a suitable dose of atropine when secretion is already paralyzed, it is clear that the nerve impulse still reaches the cell and that, therefore, atropine acts not on the nerves but on the effectors. This localization can be corroborated, moreover, by other evidence—e.g., by the production of the chemical mediator after atropine (Loewi, *loc. cit.*).

The occasional rhythmic character of the responses to repetitive stimulation or to pilocarpine (see fig. 7 and section F) is probably not accidental. It is conceivable that the response is in these cases invariably rhythmic and that if the deflections of the galvanometer are usually smooth this is due to a composite effect from asynchronous multiple elements.

Several authors have recorded simultaneously the electric responses and the salivary secretion. We did not attempt this double recording because we do not believe that there is any method available which will give even approximately accurate time relations for the beginning of secretion.

Since a single shock applied to the chorda tympani gives a definite electric response, and since this electric response is associated with secretory activity, the parasympathetic innervation of the submaxillary is not an "iterative" system (see Lapicque and Meyerson, 1921). For different reasons Rosenblueth (1932) came to the same conclusion. That single shocks applied to the sympathetic failed to demonstrate any electric responses (see section C) does not necessarily mean that there are none; the electrical phenomena might be too small for detection with the amplifications used.

SUMMARY

The electric responses of the submaxillary gland of the cat to stimulation of the chorda tympani and the cervical sympathetic nerves were recorded. The action of pilocarpine and atropine was also studied.

Single shocks applied to the chorda elicit a complex response (fig. 1) in which two components may be distinguished, a quick (fig. 2) and a slow (fig. 3).

Repetitive stimulation of the chorda produces summation of each of the two components separately (figs. 4 and 5). The response to tetanic stimulation may be rhythmic (fig. 7D).

Single shocks on the sympathetic did not cause any perceptible electrical disturbance (section C). Tetanic stimulation gives a picture similar to that obtained from the chorda (fig. 6).

Pilocarpine produces variable results (section F). The response may be rhythmic (fig. 7B). The gland is refractory to nerve stimulation while under the influence of the drug.

Moderate doses of atropine, sufficient to paralyze secretion, abolish the quick component of the response to chorda stimulation, while the slow effect persists. Larger doses abolish all electric responses.

The electrical phenomena recorded are correlated with the secretory process. An excitatory component probably does not appear in the records (see p. 515).

It is shown that atropine acts on the effector (p. 514).

The parasympathetic innervation of the submaxillary is not an "iterative" system (p. 516).

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THE ACTION OF THYROXIN ON TISSUE RESPIRATION[1]

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Within recent years several investigators have reported positive results favoring the direct action of thyroxin on tissue metabolism (Rhorer, 1924; Wohlgemuth and Klopstock, 1926; Foster, 1927; Reinwein and Singer, 1928; Weil and Landsberg, 1929; Dye and Maughan, 1929; Hopping, 1931; Gerard and McIntyre, 1933). Volumetric measurements of the oxygen consumption of tissues from thyroidectomized, from thyroxinized, and from normal animals have given variable results. More convincing evidence is given by Markowitz and Yater (1932) who have shown that the action of thyroxin on cardiac muscle is demonstrable in two-day old embryo-chick hearts before innervation of this organ has taken place. Similarly, the work of Hopping (1931) seems conclusive in showing that the oxygen consumption of alligator's blood (cells without innervation) may be increased 150 to 190 per cent above the normal by *in vivo* thyroxin injections when made 3 days premortal, while direct *in vitro* additions of the same hormone to normal blood of the same animal were without effect. Opinions differ as to the nature of this local action. The recent literature has been adequately reviewed by Gerard and McIntyre (1933) and need not be mentioned here.

The present investigations were begun in an attempt to determine not only the relative respiratory capacities of tissues from thyroidectomized, thyroxinized, and normal animals, but to study further their relative survival periods together with the nature and course of the respiratory curves.

METHOD. Six pups were thyroidectomized (method of Dot) at the age of 3 weeks and were permitted to survive for 3 months. Littermates of the same sex were kept as controls. They were killed by bleeding from the carotid artery, without anesthesia. Other pups of approximately 3 months of age were given thyroxin (Squibb's tablets) by mouth each morning 1 hour before feeding. Of these, 4 were given 4 mgm. daily for 8 to 12 days, 3 others were given 2 mgm. daily for one week, 4 mgm. daily for a second week, and 6 mgm. daily for 3 weeks, or a total of 168 mgm. each in 35 days.

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These animals showed rather marked hyperthyroid symptoms. On the days of the tissue-respiration experiments they were lightly amytalized for 30 minutes after which they were bled from the carotid and the tissues obtained at once.

Muscle fasciculi were prepared from the left semimembranous muscle, which was kept moistened with buffered saline, according to the method of Richardson, Shorr, and Loebel (1930). The oxygen consumption of these muscle strips was measured volumetrically by means of differential micro-respirometers (Fenn). These were shaken gently in a water bath, temperature 38°C. ± 0.01. Three different suspension fluids were employed for specific purposes. A non-substrate fluid contained 8.1 per cent NaCl buffered to a pH of 7.35 with M/75 phosphate mixture. Substrate suspension fluids were prepared by adding to this solution either 0.9 mgm. sodium lactate per cubic centimeter or 0.2 per cent of glucose. These solutions were kept sterile by boiling, the glucose being added just before using. One cubic centimeter of suspension fluid was added to both the tissue and differential flasks of each respirometer. The tissue strips were transferred to these flasks as prepared, care being taken to choose only those fasciculi which were uniform and not more than 0.9 mm. in diameter. Five drops of N NaOH solution were placed in the small compartment to absorb the carbon dioxide, the tissue flasks were then completely flushed with pure oxygen and the respirometers assembled in the water bath.

This entire procedure required an average time of 35 minutes. Thirty minutes were allowed for temperature equilibration before the first reading was taken. The tests were run in duplicate or triplicate, and for 4 hours in the first and 7 hours in the second series of experiments. After the runs the tissues were dried to a constant weight at 110°C. The results are expressed in cubic millimeters of oxygen consumption per milligram of dry tissue per hour. In each case other samples of the same muscle were dried in the same manner to determine the moisture content.

Influence of fasting. Fasting of the animals for 36 hours before sacrificing results in a decrease of about 21 per cent in the oxygen consumption of the isolated muscle strips when compared with that for non-fasting animals. A similar difference has previously been reported by Richardson and co-workers (1930). Without controlling this factor the oxygen consumption of the tissues from various controls may vary as much as the difference between control and experimental animals. Furthermore, the individual deviations from the mean of any given group were smaller in fasted animals. The above facts held true whether or not lactate was added as a substrate.

Influence of thyroidectomy. Whether fasting or not the oxygen consumption of muscle strips from thyroidectomized pups is reduced 24.2 per cent in the non-substrate saline and by 24.8 per cent with lactate while by only

20 per cent when glucose substrate was used. For these three solutions and for normal animals, the values were 2.73 cmm., 2.82 cmm., and 2.77 cmm. for saline, lactate, and glucose, respectively; and for cretin animals, 2.06 cmm., 2.12 cmm., and 2.21 cmm., respectively. The above data seem to indicate that muscle tissue from thyroidectomized animals is able to handle glucose better than lactate. An increase in oxygen consumption of 4 to 9 per cent is obtained by the addition of lactate or glucose to the suspension fluid in the concentrations employed. Although the oxygen consumption was relatively low in cretin animals' tissue, the gradient of decline from hour to hour was less steep. This point is of interest in the light of the opposite results obtained for thyroxinized animals and will be discussed below.

Influence of in vivo thyroxin treatment. With small daily doses of thyroxin and for treatments extending for 8 to 12 days, the results were variable from animal to animal. In some animals striking increases in tissue respiration resulted with variable rates of decline while in others there were no appreciable effects. With longer treatment, however, and with larger doses, the results were uniformly significant. Under these conditions the average figures were 4.07 cmm. and 1.55 cmm. for the first and seventh hours as compared with 2.71 cmm. and 1.28 cmm. for the same periods for the controls. These figures show an increased oxygen consumption in favor of the thyroxinized tissue of 50 per cent for the first hour and one of 21 per cent for the seventh hour. The oxygen consumption of surviving thyroxinized muscle strips is not so well maintained as that of normal animals' muscle. A graphic picture of this fact may be seen from figure 1. During the seventh hour normal muscle was respiring at a rate of 47 per cent of that obtained for the same tissue for the first hour, thyroxinized muscle strips were respiring only 38.6 per cent in the same sense, or a decline per hour of 7.5 and 8.1 per cent, respectively.

The increase in oxygen consumption in thyroxinized tissues may be due to one or both of two causes, namely, to an increase in oxidation catalysts within the tissue or to an increase in the available substrate. The more rapid decline in the oxygen consumption of thyroxinized animals' tissues may be due to first, a more rapid loss of activity of the tissue catalysts as death proceeds, or second, and what seems to be more probable, to an exhaustion of the available substrate to be burned. With this in mind similar tests were performed employing lactate as a substrate. The results for both normal and thyroxinized animals' muscle strips are given in figure 2.

The general forms of the two graphs are much the same, but closer analysis will show that with lactate the values are slightly higher and the gradients less steep. During the seventh hour the oxygen consumption of the control tissue was 57 per cent of that for the first hour, that for the

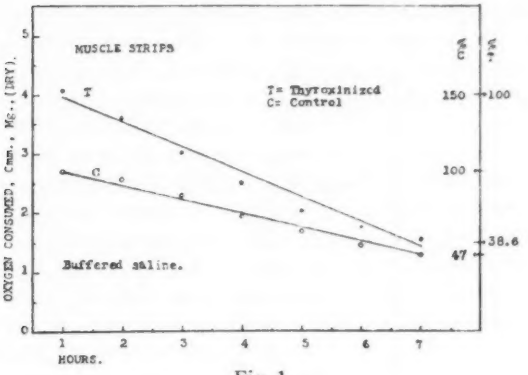

Fig. 1

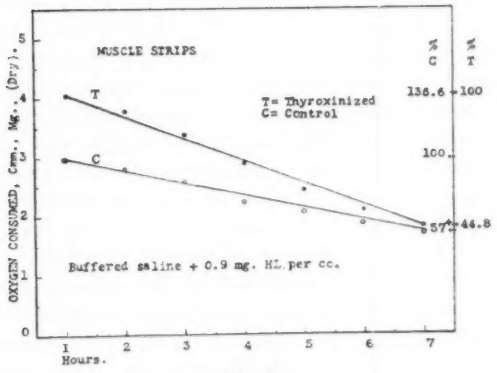

Fig. 2

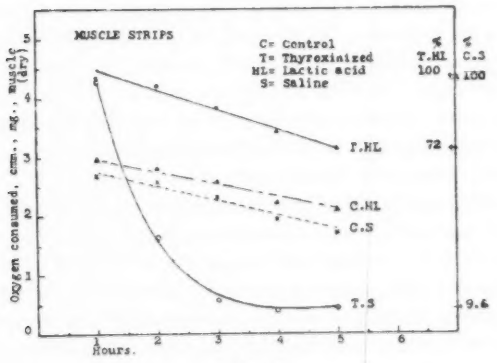

Fig. 3

thyroxinized tissue 44.8 per cent in the same sense, or hourly declines of only 6 and 7.9 per cent, respectively. When one compares the control gradient in figure 2 with each of the gradients shown in figure 1, there is an increased oxygen consumption of 8.9 and 32.8 per cent for the first and seventh hours which may be ascribed to the presence of lactate as a substrate. For the tissues of thyroxinized animals these percentages are 0 and 16.1, respectively. The steepness of the decline in oxygen consumption is determined, in part at least, by the available substrate since lactate diminishes it—and to a greater extent in normal than in thyroxinized tissue. But if it were possible to increase the rate of oxygen consumption of normal tissue to a level of that during the first hour for thyroxinized muscle by adding artificial substrates, it would seem highly improbable that the decline from hour to hour would conform to the thyroxinized tissue gradient since added substrate diminishes the rate of decline of normal tissue which, without it, would have a less steep gradient than that for thyroxinized muscle.

If the rate of oxidation of muscle metabolites in general conforms to the observation of Long (1926) that the oxidative removal of lactic acid in muscle is approximately proportional to the square of its concentration, the gradient should not be linear but in the form of a curve. That the tissue metabolites, which serve as the sole substrate when saline is used, influence the rate of oxygen consumption in approximately this ratio is strongly indicated by the curves shown in figure 3. The lines *C.S* and *C.HL* represent the averages for normal muscle strips in saline and lactate, respectively. Curve *T.S* represents the oxygen consumption curve without substrate for thyroxinized muscle strips obtained from a single animal, line *T.HL* represents the comparable oxygen consumption rate of identical strips prepared consecutively from the same muscle and same animal, but which were run in lactate substrate. These tests were run in duplicate. Curve *T.S* intersects line *C.S* during the first hour and comes to a rather low gradient by the fourth hour. The decline was 61.7 per cent for the first hour and 25.5 and 4.8 per cent for the second and third hours, respectively.

The results seem to be explained best by making two assumptions: 1, thyroxin treatment leads to an increase in the oxidative capacity of muscle tissue, either by increasing the amount or potency of its tissue oxidases or probably by serving the rôle of a co-enzyme or transportase, and 2, due to this increased oxidative capacity the available substrate is exhausted more rapidly. The probability of the substrate factor also playing a part, at least during the initial hour, has not been entirely excluded, but these experiments would indicate that it is of secondary importance.

The water content of muscle and thyroid gland was slightly, though probably not significantly, decreased in thyroxin fed animals, the averages

being 76.5 and 77.8 per cent for muscle and 71.2 and 75.3 per cent for thyroid, respectively.

At least three factors must be considered in any oxidation-reduction process, namely, the catalysts, the substrates, and the oxygen supply. In the above experiments the last factor was satisfactorily controlled, the second partially so, while the first remained a variable. That thyroxin action is not one of a direct catalyst, as suggested by Plummer and Boothby (1924), would seem to have been given considerable support from various sources. If thyroxin does act as a direct catalyst, it is difficult to explain its very slow period of induction. Absolute proof of such an hypothesis can come only from a careful study of isolated oxidase systems in which both oxygen and substrate supplies can be controlled and in which a single and specific substrate is employed.

These conditions have been approximated in the work of Adler and Lipschitz (1922) and Neuschloss (1924) who used the dinitro-benzine method of Ahlgren (1925) with Thunberg's methylene blue method, and of Dye and Maughan (1929) who employed washed minced muscle to which succinic acid was added as a substrate. The first two are substitution methods in which the artificial substrates act as hydrogen acceptors and hence measure the relative activity of the dehydrases, the last method employs a specific dehydrase system in which the dehydrase is the only variable. Use of the indophenol oxidase reagent is similarly used as a substitution method in which the reagent acts as a direct oxygen acceptor, hence should indicate the relative activity of the oxygen activator systems of the tissues. Dye and Waggener (1928) have demonstrated that tissues from thyroidectomized pups have a 10 to 50 per cent decrease in this enzyme system as compared with normal animals' tissue.

SUMMARY

Thyroidectomy in young pups leads to about a 25 per cent decrease in the oxygen consumption of surviving muscle strips. On the other hand, daily administration of rather large doses of thyroxin over rather long periods may lead to a 50 per cent increase in the oxygen consumption of similar tissues. The gradient of decline of oxygen consumption of these surviving muscle strips is steeper in the tissues of control animals than in those of thyroidectomized animals, and still steeper in tissues from thyroxinized animals. The gradient of decline together with the initial rate of oxygen consumption is interpreted as indicating the probable nature and action of thyroxin locally upon the tissues. It is considered that an excess of thyroxin leads to an increase and thyroid removal to a decrease in the amount, potency, or effectiveness of the cell respiratory catalysts and that the hormone does not serve the rôle of an independent catalyst.

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THE SHERRINGTON PHENOMENON

IV. A Study of Some of its Possible Antagonists[1]

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When the somatic motor fibers to mammalian skeletal muscle are degenerated, stimulation of the motor nerve produces contracture in the muscle. The response of the hind-limb musculature to this type of stimulation is known as the Sherrington phenomenon (1). The literature pertaining to this type of response has been reviewed recently by Hinsey and Gasser (2, 3), Gasser (4), Dale (5), and Bremer (6). More recent contributions to this general field have been made by Dale and Gaddum (7) and by von Euler and Gaddum (8). It has been shown that this phenomenon appears when the stimulus is strong enough to bring the C-spike into the action potential (3). The suggestion has been made that it may be caused by the peripheral formation of some chemical substance which has no effect on the normal muscle but does affect the sensitized degenerated muscle (2, 3, 5, 6 and 9). Dale (5) believes this substance is acetylcholine. We thought that a study of the fatigue reactions, drug antagonisms and the effect of stasis of the circulation might throw some light on this problem. The early results of this investigation were published in abstract form in 1929 (10) and they have been continued from time to time since then.

Method. In cats, the dorsal and ventral roots of the spinal nerves supplying the right gastrocnemius were cut intradurally and from 11 to 37 days' degeneration time was allowed. Most of the experiments were done under ether anesthesia but in some decapitate preparations were made. The right gastrocnemius with its blood supply intact was prepared for recording isometric contractions (Dale and Gasser, 11). The Sherrington phenomenon was produced by applying strong, rapid, alternating induction shocks to the peripheral end of the cut sciatic nerve. Maximal tetanic contractions were elicited by alternating induction shocks applied through silver wire electrodes coated with silver chloride. The anode transfixed the Achilles tendon and the two cathodes were inserted through the belly of the muscle at different angles so as to insure stimulation of most, if not

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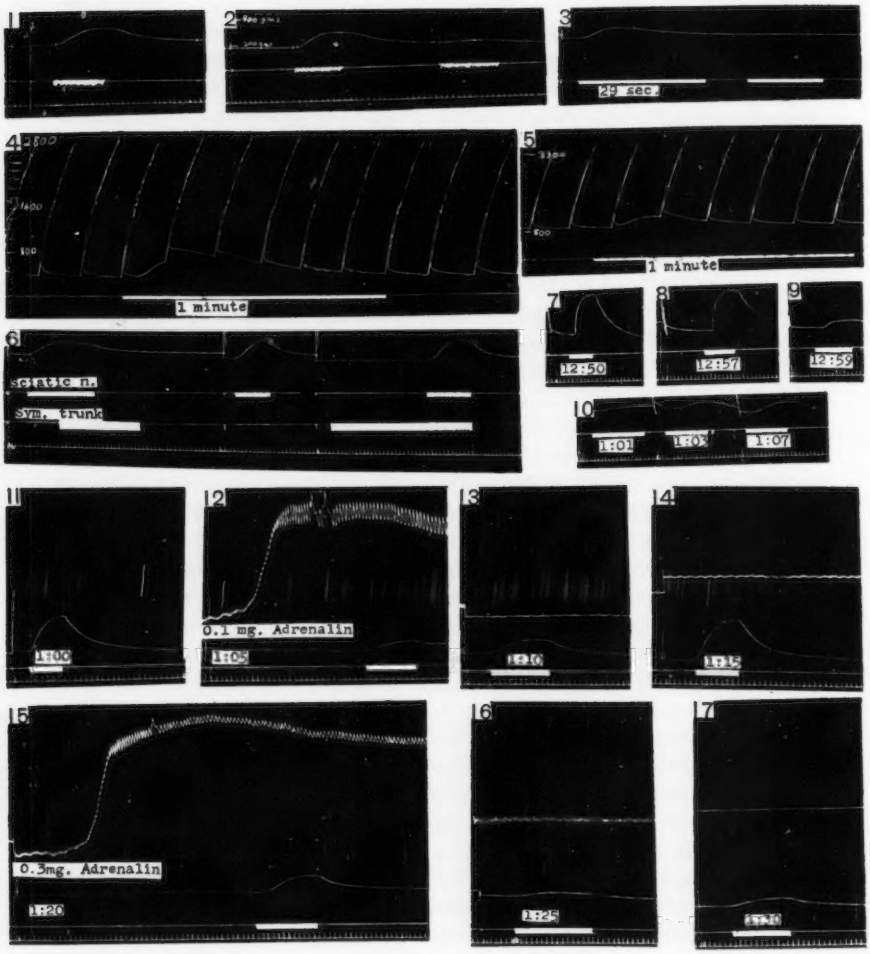
all, of the muscle. The duration of stimulation for these maximal tetanic contractions was maintained uniformly by the use of a pendulum interruptor, which swung through mercury, making its contact with a platinum point. If all of these factors were kept constant, this insured stimuli of equal strength and length, thus making possible the comparison in the same muscle of different maximal tetanic contractions of short duration. The spring used in recording the contractions was a weak one because it was necessary to obtain a Sherrington response of sufficient magnitude that it would be possible to study its different phases. It was calibrated and thus the tension in grams developed in the contractions could be determined.

The muscles were kept warm and moist by the perfusion of a warm normal saline solution at 37° over their surfaces. The bath through which the saline solution passed was maintained at a constant temperature by an electric warming plate kept under control by a thermostat.

Observations. Heidenhain (12) found that there were a number of differences between the pseudomotor contraction of the tongue and the contraction produced by stimulation of the hypoglossal nerve in the normal tongue. We have been able to confirm similar differences between the Sherrington contraction and that seen in normal limb muscle. The former has a comparatively long latent period (up to 1 second), and during the contraction phase it reaches its maximum very slowly (fig. 1). Under constant stimulation, it does not maintain the maximum tension but relaxes slowly to the base line (fig. 3). This slow relaxation is characteristic of the Sherrington phenomenon wherever it occurs, whether the stimulus is maintained throughout or ceases at the height of the contraction. The curve is a much different one from that of direct tetanus of long duration in the same muscle; this has a much shorter latent time, contraction time and a quick relaxation upon cessation of stimulation. One might explain the slow ascent in the Sherrington response by saying that here a few muscle fibers are contracting against the inertia of the remaining muscle mass. However, when just a few motor fibers to the gastrocnemius muscle are left undegenerated, the response (coil separation 9 cm.) shows a characteristic short latent period, contraction phase and relaxation time. The slow relaxation time is difficult to explain on the basis of the ordinary contractile mechanism for one would expect the muscle to relax just as rapidly in the Sherrington phenomenon as after direct stimulation, unless there is some repetitive "after-discharge" phenomonen taking place in the nerve. It is inconceivable that such a repetitive discharge could last several seconds (15 to 30). Furthermore our records show that the contraction tension was maintained from one to two seconds after cessation of stimulation before relaxation began. This curve of contraction may be best described by saying that it resembles the drug contractures seen in denervated skeletal muscle (11, 13).

The effect of fatigue. The Sherrington phenomenon renders the muscle refractory to the same type of stimulation for varying periods of time. In figure 3 a stimulation of 29 seconds' duration applied to the sciatic nerve evoked a response in the gastrocnemius muscle of 540 grams while the same stimulus applied 9 seconds later failed to call forth any response, the muscle being absolutely refractory. In figure 2 there is some recovery after a longer interval. The muscle may require up to 3 to 5 minutes to recover its irritability and in some instances even longer. With the irritability to direct stimulation remaining approximately the same, the Sherrington phenomenon may be markedly reduced in the late stages of the experiments. It is not due to local injury at the point of stimulation because it can be shown that the fatigue is still present when the stimulating electrode is moved to a fresh portion of the nerve. This may be due to a slow recovery of the nervous pathway or it may be due to the fact that, in the Sherrington phenomenon, some substance is produced which depresses the irritability to the same stimulation for some time. However Dale and Gaddum (7) were inclined to attribute a similar fatigue seen in the pseudomotor contracture in the tongue as "due mainly to a weakened efficacy of the nerve impulses in liberating the stimulant substance, at such a rate that it reaches the muscle fibres in effective concentration."

One of the main objects of this investigation was to determine whether or not the Sherrington contraction fatigued or inhibited responses to direct stimulation of the muscle. The maximal tetanic contractions were recorded at intervals of 10 seconds and were each elicited by stimuli of the same duration. Due to the fact that in some cases the muscles were quite atrophied, and that a weak spring was used so the contractions were not strictly isometric, these excursions were not large in many instances. After 4 to 6 contractions had been produced, the sciatic nerve was stimulated to produce the Sherrington phenomenon. The maximal tetanic contractions were produced throughout the Sherrington phenomenon, and for a period of time afterwards. The stimulation of the sciatic nerve was maintained for varying intervals of from one to three minutes. Figures 4 and 5 are records found typically in 10 different animals. In most cases, the maximal tetanic contractions developed slightly higher tensions at the height of the Sherrington contraction than before it. The most logical explanation for this would seem to be that at the height of the Sherrington contraction, the muscle is contracting against a greater initial tension and consequently would develop a greater contraction tension during direct tetanic stimulation. Following the relaxation and fatigue of the Sherrington contraction, there was an absence of significant fatigue to direct stimulation, in fact in most instances the tensions were essentially equal in magnitude to those obtained before the nerve stimulation. If there had been a marked fatigue due to stimulation of the sciatic and then a subsequent recovery, it might have



Figs. 1–17

Fig. 1. Sherrington contraction. Gastrocnemius muscle, sciatic nerve stimulated, Harvard coil, 6 cm. separation, rapid faradic. Cat 2, 6-7L and 1-2-3S dorsal and ventral roots sectioned, 24 days' degeneration.

Fig. 2. Fatigue by the Sherrington response to the same type of stimulation through sciatic nerve, 6 cm. coil separation, cat 2.

Fig. 3. Complete inhibition of subsequent Sherrington contraction. Sciatic nerve, 6 cm. coil separation. Cat 3, 6–7L and 1-2-3S dorsal and ventral roots sectioned; 18 days' degeneration.

Fig. 4. Absence of fatigue by Sherrington response to short direct tetanization.

been argued that a chemical substance had been elaborated due to the nervous stimulation which had fatigued the muscle to direct stimulation, such as Gasser and Dale (13) have shown for acetylcholine, nicotine and trimethylsulphonium iodide. If a chemical substance is formed peripherally, it must be one which does not markedly depress the irritability of the muscle to direct stimulation. If Dale's contention that it is acetylcholine is true, the acetylcholine in the tissues does not behave as it does when injected.

Gasser and Dale (13) have shown that after fatigue of the muscle by direct stimulation, the acetylcholine contracture is obtainable undiminished. Immediately following long fatigue of the muscle by direct tetanization, it may be impossible to elicit any Sherrington contraction whatsoever (4 instances), it may be present but diminished in height (7 instances), or it may be unchanged (4 instances). It is conceivable that there should be some fatigue of the Sherrington phenomenon in most cases following long tetanization, because the direct stimulation is applied directly to the muscle and may bring into activity some of the nerve fibers responsible for the Sherrington contraction. This would be especially true in case a strong stimulus were used in the direct stimulation. It might also be that the threshold of the nerve fibers in the intramuscular twigs may be lower than in the degenerated nerve trunk where the large amount of non-nervous tissue probably interferes with the reception of the stimuli

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Faradic stimulation to muscle, Harvard coil 6 cm. coil separation. Faradic stimulation of sciatic nerve, coil separation 6 cm. Cat 38, 6-7L and 1-2-3S dorsal and ventral roots sectioned, 34 days' degeneration.

Fig. 5. Absence of fatigue by Sherrington response to short direct tetanization. Stimulation same as in figure 4. Cat 39, 6-7L and 1-2-3S dorsal and ventral roots sectioned; 35 days' degeneration.

Fig. 6. Concomitant stimulation of the lumbar sympathetic trunk above the 5L ganglion and the unsectioned sciatic nerve. Rapid stimulation from Harvard coils, both coil separations 6 cm. Cat 242, 4-5-6-7L and 1-2-3S dorsal and ventral roots sectioned; 18 days' degeneration.

Figs. 7, 8, 9, 10. Contractions recorded in the gastrocnemius on stimulation of the sciatic nerve, 5 cm. coil separation. Cat 204, 3-4-5-6-7L and 1-2S dorsal and ventral roots sectioned; 14 days' degeneration.

Fig. 7. Response at 12:50 p.m.

Fig. 8. Aorta clamped above its bifurcation, both hypogastrics and right external iliac arteries clamped, 10 seconds before this contraction was recorded.

Fig. 9. Response at 12:59.

Fig. 10. Response obtained at 1:01 and circulation was immediately reëstablished.

Figs. 11, 12, 13, 14, 15, 16, 17. Blood pressure tracing from the carotid artery in decapitate preparation. Contractions from the gastrocnemius on stimulation of the sciatic nerve, 6 cm. coil separation. Cat 233, 4-5-6-7L and 1-2-3S dorsal root ganglia removed and corresponding ventral roots sectioned; 16 days' degeneration. Osmic acid sections show no myelinated fibers in tibial nerve. Crystalline adrenalin in normal saline. No blood pressure tracing in figure 17 due to clot.

by the small nerve fibers. The chance distribution of the silver wire electrodes near intramuscular nerve twigs would also lead to variability in fatigue to the Sherrington phenomenon by the direct stimulation of the muscle. However, it may well be that fatigue of the muscle fibers themselves to direct stimulation may also fatigue them to stimulation through the partially denervated sciatic nerve; these experiments would not give conclusive proof in that direction.

Effect of stimulation of the lumbar sympathetic chain. Figure 6 shows the results obtained by concomitant stimulation of the sympathetic trunk (above the 5L ganglion) and the sciatic nerve after the 4-5-6-7L and 1-2-3S dorsal and ventral roots had been sectioned. The sciatic nerve had not been sectioned peripherally. This shows that the contraction of the gastrocnemius is not inhibited by a stimulation of the sympathetic trunk which produced erection of the hairs in the areas of distribution of this part of the trunk. Similar results were found in the quadriceps muscle in this same preparation. We have not been able to get any evidence for inhibition of the Sherrington phenomenon such as Dale and Gaddum (7) obtained for acetylcholine by concomitant stimulation of the sympathetic trunk. Ginezinski and Orbeli (14) found a facilitating effect upon the Vulpian phenomenon in the tongue by stimulation of the cervical sympathetic.

Effect of stasis of circulation. Lewis (15) found that, if the circulation was arrested, the vasodilatation due to the stimulation of sensory fibers did not occur until the circulation was released. He thought a substance was probably formed in the skin which was not allowed to reach its point of action until the blood could be free to carry it there. We thought that there might be some parallelism between the vasodilatation in the skin and the Sherrington phenomenon in regard to the effect of circulatory arrest; possibly that it would not occur when there was a stasis of the vascular supply. We have recorded the maximal tetanic contractions and the Sherrington phenomenon. Then five minutes later (time allowed to recover from the fatigue) the blood supply to the muscle was occluded. After varying intervals, the Sherrington response and the maximal tetanic contractions were again recorded. Then attention was paid to see if release of the circulation caused any increase in tension.

Figure 8 shows the presence of the Sherrington contraction 10 seconds after clamping the aorta just above the bifurcation, ligature of both hypogastric arteries and clamping the right external iliac artery. The records shown in figures 7, 8, 9 and 10 are typical of those obtained in four other experiments. From our observations we were convinced that the Sherrington phenomenon was distinctly present after the arterial blood supply was occluded, and within the first 10 to 20 seconds it was not diminished in amplitude. With an interval of two minutes or more after the arresting of the arterial blood supply, the tensions developed both by the direct

and indirect stimulation of the muscle were considerably diminished. This was to be expected inasmuch as a decrease in irritability of the muscle should be present in the absence of the blood supply. It is inconceivable that a collateral blood supply could have been present in sufficient magnitude to explain these results. The decrease in irritability with longer intervals certainly proves that there could not been have any considerable collateral circulation. No contractions were seen in the muscles upon release of the circulation.

These observations can be taken to mean that the Sherrington contraction is present after occlusion of the arterial blood supply. Heidenhain (12) found that removal of the blood supply did not interfere with the pseudomotor reaction in the tongue, in fact this response was present after excision of the tongue from the body. These findings are similar for the limb muscles. If a chemical substance is produced by the nerves which are left to those muscles, it does not depend solely upon the blood stream for its transport and has ready access to the muscle fibers themselves.

Drug antagonisms. Inasmuch as the pseudomotor reaction in the tongue and the Sherrington phenomenon have been thought to be parasympathetic responses (16, 17), we have investigated a few of the drug reactions. Hinsey and Gasser (2) found that 1 cc. of 1/1000 adrenalin completely inhibited the Sherrington phenomenon. Dale and Gaddum (7) have failed to find complete inhibition but rather incomplete inhibition followed by a facilitation of the Sherrington response. On the basis of their observations of the facilitation of the acetylcholine contracture on isolated strips of denervated muscle of the diaphragm, they attribute the facilitation of the Sherrington phenomenon to the adjuvant action of adrenalin to a chemical substance liberated by the nerve endings which reaches the denervated muscle fibers directly through the intervening lymph. They suggest the possibility that the inhibition is due to a reduced permeability of the capillary walls for the portion of the chemical substance which first has leaked into the blood and then secondarily diffuses through the capillary walls. However, a reference to figures 7 and 8 will show that not a great portion of the Sherrington phenomenon can be due to an active blood supply or it would not be present undiminished following a statis of the arterial blood supply. In our earlier published observations, we used a prepared solution of adrenalin chloride, which also contains chloretone. Furthermore we injected rather large doses (1 mgm. of adrenalin). In a subsequent series of five animals, we have injected 0.1 to 0.3 mgm. of crystalline adrenalin in normal salt solution intravenously. We are indebted to Parke, Davis & Co., who generously supplied the crystalline adrenalin. With this dosage, we have always seen partial inhibition, never complete. Even with dosages of 1.0 mgm., the inhibition was not complete. We have never seen an adjuvant action in any of our preparations, even when we have used

crystalline adrenalin and when we have stimulated at various intervals following the injection. Figures 11, 12, 13, 14, 15, 16 and 17 illustrate the partial inhibition

The injection of 2 cc. of a 1/1000 solution of pilocarpine hydrochloride into the external jugular vein caused no response in the partially denervated gastrocnemius. Instead of increasing the response to nerve stimulation, it antagonized it as Langworthy (18) found for the pseudomotor response in the tongue. The intravenous injection of atropine sulphate and of scopolamine hydrobromide exerted no marked inhibitory effect on the Sherrington response. The following abstracts from the analyses of records illustrate this point:

Cat 36

1:15 Sherrington response was 730 grams.
1:20 1 cc. of 1/1000 atropine sulphate injected into the external jugular vein.
1:25 Sherrington response was 730 grams.
1:35 Sherrington response was 760 grams.
1:40 1 cc. of 1/1000 atropine sulphate injected into the external jugular vein.
1:45 Sherrington response was 700 grams.
1:55 Sherrington response was 705 grams.
2:10 Sherrington response was 790 grams.
2:35 Sherrington response was 700 grams.
2:40 1 cc. of adrenalin chloride (solution) into left femoral vein.
2:45 No Sherrington response obtainable.

Cat 37

12:45 Sherringron response was 600 grams.
12:48 1 cc. of 1/1000 scopolamine HB_r injected into left femoral vein.
12:52 Sherrington response was 590 grams.
12:55 Sherrington response was 590 grams.
1:05 Sherrington response was 640 grams.
1:10 Sherrington response was 520 grams.
1:18 1 cc. of 1/1000 scopolamine HB_r again injected into left femoral vein.
1:25 Sherrington response was 580 grams.
1:30 Sherrington response was 485 grams.
1:33 $\frac{3}{4}$ cc. adrenalin chloride (solution) injected into left external iliac artery which was so clamped off that the drug would be delivered to the right external iliac artery.
1:35 Sherrington response not obtainable.

It is evident that a parasympathetic-augmenting drug like pilocarpine did not produce a contracture similar to the Sherrington phenomenon and parasympathetic inhibitors like atropine and scopolamine did not inhibit it. It is quite clear that the Sherrington phenomenon cannot be put into the classification of a parasympathetic response. As Gasser and Dale (13) have pointed out, "These antagonisms can only be used properly for such specific indications when they are produced by very small doses." Frank and his co-workers have used doses of scopolamine in studying antagonisms which

we feel are far too great to render their results interpretable on the basis of known facts about the parasympathetic drugs. It is of interest to note that Bremer (19) found that the dose of atropine abolishing neuromuscular contracture is much higher than that suppressing parasympathetic effects.

Similar to Heidenhain's findings for the pseudomotor reaction in the tongue, we found that curare inhibits the appearance of the Sherrington phenomenon.

Cat 39

11:35 Sherrington response was 620 grams.
11:40 1 cc. of a 1 per cent extract of curare injected into the left femoral vein.
11:49 No Sherrington response was obtainable at a time when the opposite motor nerve was blocked.

We were able to confirm the observations of Dale and Gasser (11) on histamine in that we found that the injection of 2 cc. of 1/1000 histamine (ergamine acid phosphate) into the external jugular vein produced no reaction in the partially denervated right gastrocnemius.

CONCLUSIONS

1. The Sherrington response renders the gastrocnemius refractory to subsequent stimulation for about 10 seconds. After 15 seconds it is partially refractory and gradually recovers its irritability. It does not fatigue the muscle to direct stimulation. Fatigue of the muscle by direct tetanization may produce complete, partial or no inhibition to the Sherrington response.

2. Stimulation of the abdominal sympathetic trunk before or during the Sherrington contraction does not inhibit it.

3. Stasis of the arterial blood supply does not prevent the occurrence of the Sherrington phenomenon, in fact it may not inhibit it at all in the first 10 seconds.

4. Crystalline adrenalin (0.2 to 0.3 mgm. and 1.0 mgm.) inhibits, but not completely so, the Sherrington response.

5. Pilocarpine does not produce a contracture in denervated muscle, in fact it inhibits the Sherrington response.

6. Atropine SO_4 and Scopalimine HB_r do not inhibit the appearance of the Sherrington phenomenon.

7. Curare inhibits it.

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THE SHERRINGTON PHENOMENON

V. Nervous Pathways[1]

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It has been shown that in order to sensitize the skeletal muscles to the Sherrington phenomenon, it is only necessary to section the ventral roots of the spinal nerves which supply the respective muscles (1). It has been assumed since Sherrington (2) first described this peculiar reaction that it is produced by antidromic conduction over the somatic sensory fibers supplying the muscle. Attention was called to the similarity of this response to dorsal root vasodilatation (3 and 4). It was pointed out independently by Dale (5), Bremer and Rylant (6) and Hinsey and Gasser (4) that both the vasodilatation and the Vulpian-Heidenhain-Sherrington phenomena are probably due to the elaboration of some chemical substance at the terminations of the sensory fibers. Gasser (7) has thoroughly reviewed the literature pertaining to this problem.

However it is necessary to consider in some detail the observations which rule out the thoracolumbar sympathetic fibers from participation in the Sherrington response. Sherrington (2) showed that it was not obtained when the dorsal root ganglia were removed as well as the ventral roots sectioned. The number of experiments and the details were not stated. Van Rijnberk (8) was unable to produce it by stimulating the abdominal sympathetic trunk in two animals. He also obtained it in two animals after removal of the abdominal sympathetic trunk but he does not state specifically how much of the trunk was removed. In working with the sciatic nerve, it would be necessary that the abdominal sympathetic trunk be removed down through and including the first two sacral ganglia. No one has ever proved that it can be produced by stimulation of the dorsal roots alone or that it would occur when all of the sympathetic fibers were removed beyond peradventure. It was due to dissatisfaction with the existing evidence that the present investigation was undertaken.

Method. The observations described in this report were taken from 58 adult cats. All of the operative procedures were conducted with careful

[1] This study was conducted with the aid of a grant from the Rockefeller Foundation for Fluid Research in the Medical Sciences at Stanford University.

aseptic technique and the animals were anesthetized at the time of operation in most instances with amytal. All of the experiments were conducted on muscles in the hind limb, the quadriceps femoris (with the rectus femoris excluded) and the gastrocnemius. The lumbosacral plexus is formed in the cat from the 4-5-6-7L and 1-2S segments and the thoracolumbar sympathetic fibers are derived from the lumbar sympathetic trunk from the corresponding chain ganglia. In extirpating the sympathetic trunk, it was found possible to remove from the second lumbar chain ganglion down to below the seventh lumbar ganglion and sometimes the first sacral but the second sacral is difficult to approach. For this reason it was possible to remove all of the postganglionic sympathetic neurons giving rise to fibers in the femoral nerve which is derived from L4-5-6, but it was impossible to extirpate all of them from the sciatic nerve. The dorsal root ganglia were excised to produce degeneration of the sensory fibers. The respective nerves were fixed and sectioned both for osmic acid and pyridine silver in order to learn what fibers were still left in the corresponding nerves. It will be much more convenient to present the various operative procedures along with the observations which were made on the different phases of this problem. Careful autopsies were performed following each experiment.

Following various degeneration times (usually 14 to 18 days), the animals were anesthetized with ether and a tracheal cannula was inserted. Some of the preparations were decapitated and artificial respiration was supplied. The muscles studied were dissected free from all surrounding muscles and so fixed that only the contraction of the respective muscle would record on the kymograph. The blood supply of the muscles was left intact. Contractions were recorded by the method used by Gasser and Dale (9) but the contractions were not isometric due to the fact that a weak spring was used with the degenerated muscles. In eliciting the responses, rapid, alternating induction shocks from a Harvard coil (6–7 cm. coil separation; 2 amperes delivered to the primary coil) were applied to the distal end of the cut femoral nerve when the quadriceps was being studied and to the sciatic with the gastrocnemius. The greatest possible conducting distance was used in each case. The muscles and nerves were kept warm and moist during the progress of the experiment.

Observations. *Is the response due to nerve conduction?* It has been suggested that this response is not due to nervous conduction but merely to a spread down along the connective tissue of the nerve or possibly to conduction over the bands of Bünger. This was controlled by cutting the sciatic nerve at the sciatic notch in four cats and allowing 18 to 21 days' degeneration. In another animal, two months degeneration time was given. In none of these, even with the secondary coil directly over the primary, was there any semblance of a contraction in the muscle. To

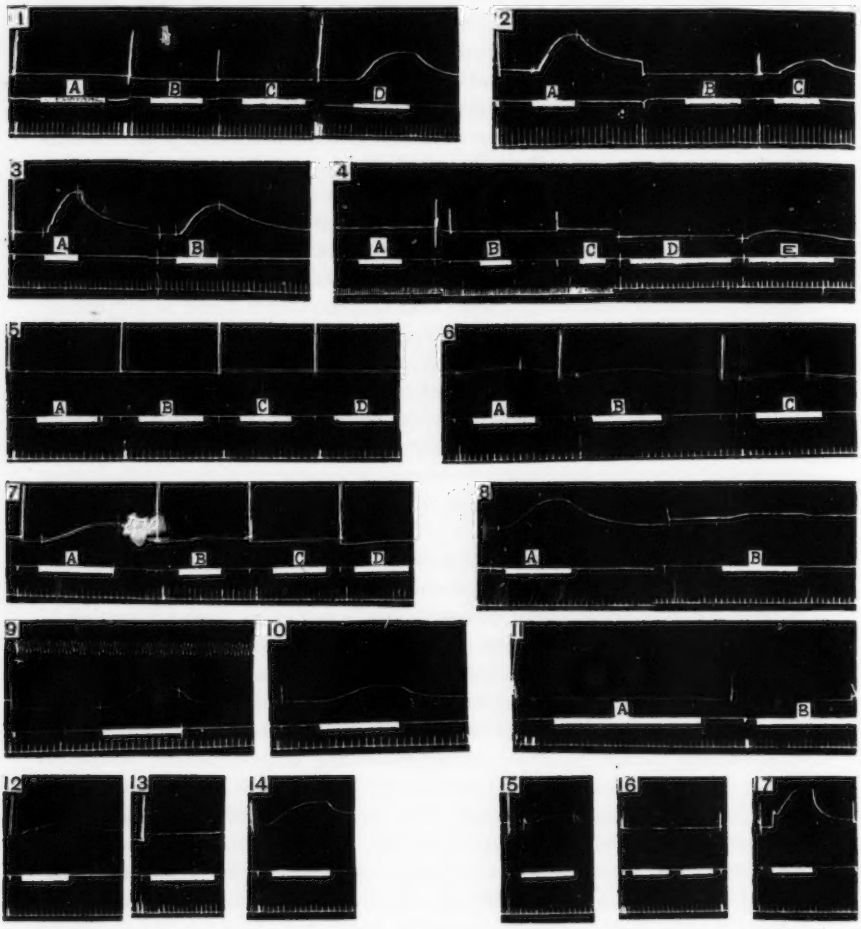
control this still further, the lumbar sympathetic trunk was removed from L3 to L7 inclusive on the right side in two cats. A month later, the 4-5-6-7L and 1-2-3S dorsal root ganglia were completely removed and the corresponding ventral roots sectioned. After a degeneration time of 13 days, the femoral nerves, which were devoid of any nerve fibers, were silent and Sherrington responses were obtained from the sciatic which contained only the postganglionic sympathetic fibers arising in the first two sacral ganglia (fig. 1).

A further control has been performed time and time again in both the femoral and sciatic nerves. The nerves were stimulated at a certain point and responses obtained; then they were crushed below the point of stimulation. The responses were always abolished but were found to be present when the point of stimulation was moved distal to the point of pinching rather than proximal to it (fig. 2). These nerves were intentionally moistened to facilitate any spread which might have occurred.

In the light of our experiences, we were convinced beyond the shadow of any doubt that the Sherrington response is due to nervous conduction and not to spread or conduction over non-nervous structures. Sherrington (2) and Hinsey and Gasser (1, 4) were led to the same conclusion for additional reasons.

Is the conduction due to somatic motor fibers? It is very important to be sure that there could not be a few somatic motor fibers remaining in these partially degenerated motor nerves. We had the anatomical control of osmic acid preparations of the respective nerves and, if there were any somatic motor nerves present, they should have appeared as intact, fairly large myelinated fibers. The femoral nerve in the cat is supplied by the 4-5-6L segments and the sciatic from the 6-7L and 1-2S segments. We have controlled this by sectioning the dorsal and ventral roots of 3-4-5-6-7L and 1-2S and found the response in the quadriceps. Furthermore we have found it in the gastrocnemius following section of the 4-5-6-7L and 1-2-3-4-5S dorsal and ventral roots (fig. 2). This would certainly rule out any atypical distribution of somatic motor fibers to the sciatic from adjacent segments. The fact that the Sherrington response was obtained in both the quadriceps (fig. 17) and the gastrocnemius (fig. 14) following total extirpation of the spinal cord from the 3L caudalward should remove any doubt as to the possibility of somatic motor fibers being the ones involved.

Is the conduction over sensory fibers? As has been pointed out, it is quite generally assumed that the sensory fibers are the ones which conduct the impulses responsible for the phenonenon. This has been tested in two ways. The lumbosacral dorsal and ventral roots were exposed and sectioned proximal to the dorsal root ganglia. After sufficient degeneration times had been allowed, the 6-7L and 1S dorsal root ganglia were exposed



Figs. 1–17

Stimulation consisted of rapid faradic stimuli from Harvard coil with 2 amperes delivered to the primary. Time in seconds.

Fig. 1. Cat 293. 1/18/32. Abdominal sympathectomy, right chain removed from 3L through 7L.; 2/24/32. Removed 4-5-6-7L and 1-2-3S dorsal root ganglia and sectioned corresponding ventral roots; 3/8/32. experiment. a. Quadriceps, femoral nerve, 6 cm. coil separation. b. Quadriceps, femoral nerve, 4 cm. c. Quadriceps, femoral nerve, secondary directly over primary. d. Gastrocnemius, sciatic nerve, 6 cm.

No normal myelinated axons were seen in cross sections of the femoral and tibial nerves.

and stimulated with strong faradic stimulation. At no time in five different experiments have we seen any evidence of the Sherrington response in the gastrocnemius from stimulation of the dorsal root ganglia or the proximal portion of the dorsal roots attached to the ganglia. This negative result was present when the phenomenon could be demonstrated by stimulation of the sciatic nerve.

Fig. 2. Cat 298. 5/5/32. Sectioned right 4-5-6-7L and 1-2-3-4-5S dorsal and ventral roots proximal to the dorsal root ganglia.; 5/27/32. experiment. a. Gastrocnemius, sciatic nerve, 6 cm. b. Sciatic nerve had been crushed below the point of stimulation and moistened with normal saline. The nerve was stimulated proximal to the point of crushing, 6 cm. No evidence of spread. c. Sciatic nerve stimulated (6 cm.) below point of crushing.

Fig. 3. Cat 299. 5/6/32. Sectioned right 4-5-6-7L and 1-2-3-4-5S dorsal and ventral roots proximal to the dorsal root ganglia.; 5/23/32. experiment. a. Quadriceps, femoral nerve, 6 cm. b. Gastrocnemius, sciatic nerve, 6 cm.

Fig. 4. Cat 212. 3/18/31. Bilateral sympathectomy, removed both chains from L2 down through L7.; 4/30/31. Sectioned right 4-5-6-7L and 1-2S dorsal and ventral roots proximal to the dorsal root ganglia.; 5/29/31. experiment. a-b-c. Quadriceps, femoral nerve. a.-6 cm.; b.-5 cm.; c.-3 cm. d-e. Gastrocnemius, sciatic nerve. d.-6 cm.; e.-5 cm.

Figs. 5 and 6. Cat 511. 8/5/32. Sympathectomized on right side from L2 through L7. S1 and S2 intact.; 8/26/32. Cut right 4-5-6-7L and 1-2-3S dorsal and ventral roots proximal to the dorsal root ganglia.; 9/10/32. experiment.

Fig. 5, a-b-c-d. Quadriceps, femoral nerve. a.-6 cm.; b.-5 cm.; c.-4 cm.; d.-0 cm.

Fig. 6, a-b-c. Gastrocnemius, sciatic nerve. a -6 cm.; b.-6 cm.; c.-5 cm.

Fig. 7. Cat 294. 1/28/32. Right abdominal sympathectomy, removed 7L through S1 (S2 is still intact).; 7/12/32. Cut right 4-5-6-7L and 1-2-3S dorsal and ventral roots.; 7/28/32. experiment, decapitate. a. Quadriceps, femoral nerve, 6 cm. b-c-d. Gastrocnemius, sciatic nerve. b.-5 cm.; c.-6 cm.; d.-5 cm.

Fig. 8. Cat 204. 2/27/31. Bilateral abdominal sympathectomy L2-L7 inclusive. 5/21/31. Sectioned 3-4-5-6-7L and 1-2S dorsal and ventral roots proximal to the dorsal root ganglia; 6/4/31 experiment. a. Gastrocnemius, sciatic nerve, 6 cm. b. Quadriceps, femoral nerve, 5 cm.

Figs. 9 and 10. Cat 234. 7/15/31 Removed 4-5-6-7L and 1-2-3S dorsal root ganglia and sectioned corresponding ventral roots.; 8/3/31 experiment, decapitate.

Fig. 9. Gastrocnemius, sciatic nerve, 6 cm. Carotid blood pressure.

Fig. 10. Quadriceps, femoral nerve, 6 cm.

Figs. 11-12-13-14-15-16-17. Cat 348. 6/23/32. Removed all of the spinal cord from 3L caudalward.; 7/29/32 experiment, decapitate.

Fig. 11, a and b. Absence of response in right gastrocnemius when right abdominal sympathetic trunk was stimulated above the 5L ganglion, coil separation 6 cm.

Fig. 12. Gastrocnemius, gray ramus of S1 stimulated, 6 cm.

Fig. 13. Absence of contraction in gastrocnemius (following section of sciatic nerve) on stimulation of gray ramus of S1.

Fig. 14. Gastrocnemius, sciatic nerve, 6 cm.

Fig. 15. Quadriceps, gray ramus of 6L stimulated, 6 cm.

Fig. 16. Absence of contraction in quadriceps (following section of femoral nerve) on stimulation of gray ramus of 6L.

Fig. 17. Quadriceps, femoral nerve, 6 cm.

In eight animals, the dorsal and ventral roots from the L4 down through S3 were sectioned and from 12 to 22 days' degeneration allowed. It was found that in every one of these, there was a Sherrington response in the quadriceps (fig. 3 A) which resembled that seen in the gastrocnemius (fig. 3 B) on stimulation of the sciatic nerve. From this it was evident that the femoral nerve and the quadriceps muscle could be used as well as the sciatic nerve and the gastrocnemius.

It was then possible to prepare a femoral nerve which should contain only sensory fibers and a sciatic nerve which should contain both sensory and postganglionic sympathetic fibers. This was done by first removing the right abdominal sympathetic chain from L2 through L7 chain ganglia. Various (sufficient in each case) intervals were given for the degeneration of the postganglionic sympathetic fibers in the femoral nerve. Inasmuch as the first and second sacral chain ganglia were not removed, the sciatic should still contain some postganglionic sympathetic fibers (their number however would be decreased by the degeneration of the postganglionic fibers arising in the 6 and 7L chain ganglia). After the respective degeneration times, the dorsal and ventral roots of 4-5-6-7L and 1-2-3S were sectioned proximal to the dorsal root ganglia. After 12 to 29 days' degeneration, it was found that four animals showed absolutely no response at all in the quadriceps with any strength of stimulation on the femoral nerve (fig. 4 A-B-C-D and fig. 5) while the gastrocnemius responded positively and definitely in every case (fig. 4 E and fig. 6). The response in the gastrocnemius was diminished in every case as might be expected from the fact that the content of postganglionic sympathetic fibers was also decreased. In one animal, the gastrocnemius was definitely positive (fig. 8 A), while once and only once a very small response was seen in the quadriceps (fig. 8 B). In this particular case, in the absence of more precise information, the possibility must be recognized that the slight contraction might have been due to antidromic conduction over sensory fibers. However, in the light of the evidence we accumulated later, we are inclined to believe that this was due to a few postganglionic sympathetic fibers which had joined the femoral nerve in some atypical manner. In another animal, the sympathetic trunk was removed from below L6 to below S1 chain ganglia. Following section of the dorsal and ventral roots and degeneration, the femoral nerve contained its normal number of both sensory and sympathetcic fibers. Here there was a very well-developed response (fig. 7 A) in the quadriceps. On the other hand, the sciatic nerve contained its normal number of sensory fibers but only the postganglionic sympathetic fibers from L6 and S2 chain ganglia. The response here was obtained only once and then very slightly (fig. 7 B).

These experiments showed us quite conclusively that antidromic conduction over sensory fibers does not adequately explain the response.

The thoracolumbar sympathetic pathways. In nine different experiments, following section of the dorsal and ventral roots to the lumbosacral plexus, the right abdominal sympathetic trunk was stimulated at different levels so that the preganglionic fibers of the pathways to the lumbosacral plexus were stimulated. That the fibers were in a good physiological state was evidenced by the erection of the hair in the tail and limbs. We have never seen any indication whatsoever of contraction in the quadriceps and gastrocnemius following such stimulation (fig. 11 A and B). However in one of the experiments, some very interesting observations were made. In cat 348, the spinal cord was completely removed from the 3L on down through the lower lumbosacral segments. The animal was kept for 16 days. On the day of the experiment, it was decapitated and the gastrocnemius and the quadriceps (without the rectus femoris) were prepared for tracings. The abdominal sympathetic chain was isolated for stimulation by dissecting away the iliopsoas muscle. The trunk was sectioned below the 4L chain ganglion. Inasmuch as there was no pathway for afferent conduction here following removal of the spinal cord and section of the sympathetic trunk above the point of stimulation, the possibility of eliciting extraneous reflexes was obviated. The possibility of spread to surrounding tissues was carefully controlled. Stimulation of the trunk above the 6L chain ganglion produced no response in the gastrocnemius (fig 11). However it was found that stimulation of the 7L gray ramus and that of the 1S (fig. 12) produced contractions in the gastrocnemius, which while smaller in extent, resembled those which were obtained directly from the sciatic nerve (fig. 14). As a control the sciatic nerve was sectioned and the gray rami were again stimulated and no contraction took place in the muscle (fig. 13). Stimulation distal to the section in the sciatic nerve produced a response (fig. 14). Observations were made in a similar manner for the gray ramus of the 6L with contractions in the quadriceps (fig. 15). Section of the femoral nerve removed the response (fig. 16) but it was still obtained when this nerve was stimulated distal to the section (fig. 17).

The significance of the postganglionic sympathetic fibers is given added weight by the fact that it was possible to obtain the Sherrington response undiminished in both the quadriceps and the gastrocnemius in eight different animals where the nerve contained only postganglionic sympathetic fibers. This was accomplished by complete removal of the dorsal root ganglia from the 4-5-6-7L and 1-2-3S segments and section of the corresponding ventral roots. After degeneration times varying from 12 to 37 days, these nerves should contain only the sympathetic fibers which pass to the peripheral nerves through the gray rami. Osmic acid preparations were made to determine the presence of any myelinated fibers whose cells of origin might have been missed at operation. In only one preparation was there any question as to the completeness of the degeneration of the

myelinated fibers. Figure 9 illustrates the response in the gastrocnemius. The accompanying blood pressure tracing shows no change during the stimulation. If a chemical substance is liberated peripherally by the sympathetic fibers, it must either be quickly destroyed or it does not affect blood pressure under the conditions of our experiments, for we have never seen any change in the accompanying blood pressure tracing which we believed to be significant. Figure 10 shows the response in the quadriceps when the femoral nerve contains only sympathetic fibers.

Inasmuch as the response is absent when the dorsal root sensory fibers are present alone and is positive undiminished when they are removed, it would seem that the sensory fibers do not play an essential rôle as the conductors of impulses responsible for this phenomenon. This deduction is also supported by the absence of responses on stimulation of the dorsal roots. Due to the fact that the phenomenon is not produced by the stimulation of preganglionic fibers, but is elicited on activation of the postganglionic ones both in the gray rami and in the peripheral nerves, it seems necessary to conclude that it is produced by impulses carried over the postganglionic thoracolumbar sympathetic fibers. This conclusion is further justified by the fact that controls have shown that the conduction is over nerve fibers and does not occur in a nerve in which the nerve fibers have been completely removed. Conduction over axons arising from scattered cells along the ventral roots is negated by the fact that these cells would not have been materially disturbed in any of our operative procedures, at least not differently so. Furthermore, the work of Windle (10) shows that these cells are too few in number and inconstant in the cat to account for the contraction seen, even if their axons could be shown to pass into peripheral nerves.

Comment. Inasmuch as the histology of the innervation of skeletal muscle (3) and its blood vessels (11) supported the possibility of antidromic conduction over the dorsal roots with elaboration of a chemical substance, we have suggested that the Sherrington phenomenon was elicited by impulses travelling over sensory fibers. This assumption was given further support by the work of Hinsey and Gasser (4) which showed that both dorsal root vasodilatation and the Sherrington phenonenon appeared when the strength of the stimulus was sufficient to bring the C-spike into the action potential. This pointed to small fibers and, inasmuch as the C-spike is present in the potential of sensory as well as visceral efferent fibers, no difficulty was introduced. Consequently these observations came as a distinct surprise in the light of our own previous observation and deductions and of those of Sherrington and Van Rijnberk. However the evidence as produced by these experiments is of such nature that we have been forced to abandon the sensory pathways as the ones utilized in the Sherrington phenomenon. With this abandonment, the last prop of

support for dorsal root efferent conduction in muscle tonus probably goes (Ranson, 12).

The absence of contraction in the sensitized muscle on stimulation of the dorsal roots would not be particularly disturbing alone. When it is combined with the other evidence it is of some significance. It might be argued that following the laminectomy, the stumps of the dorsal roots and the ganglia to which they were attached had degenerated,—possibly were traumatized—and consequently would not respond. However this may be answered by the fact that stimulation of the nerve produced the response in the same preparations. Stimulation of the ganglia themselves would not do it in spite of the fact that stimulation of the ganglia has been shown to produce vasodilatation in the limb (Ranson and Wightman, 13). Furthermore in the absence of the response when the sensory fibers were in the femoral nerve alone and its presence undiminished when all of the sensory fibers were removed from both the femoral and the sciatic nerves should without a doubt make the case fairly conclusive against the sensory fibers.

The fact that we have been unable to obtain the phenomenon on stimulation of the preganglionic fibers in the abdominal sympathetic trunk has been very baffling. This shows first of all that it cannot be due to a conduction over sensory fibers which are said to join with the sympathetic trunk in the region of the lower thoracic and upper lumbar segments to again leave it by way of gray rami at lower levels and pass in the distribution of the peripheral nerves (Kuntz and Farnsworth, 14). Secondly, if we accept the orthodox distribution of preganglionic fibers in the sympathetic trunk, it shows that there must be some block at the synapse for this response inasmuch as hair erection in the areas of distribution of the lumbosacral plexus could be produced. It may be a matter of stimulation frequency and that the rapid faradic stimulation which we used produced a synaptic fatigue. On the other hand, this rapid stimulation produced the response when applied to the gray rami and peripheral nerves. It seems unlikely that the frequency of impulses in the postganglionic fibers, following preganglionic stimulation in the trunk, is below that minimal frequency which is required by the response. Even if we were to double the refractory period of 20 sigmas in the cat sympathetic synapse, it should be possible to get through 25 impulses per second, a frequency which should produce the contracture. The other alternative would be that the preganglionic fibers carrying impulses for this type of response do not follow the typical course down the sympathetic trunk but instead pass up from lower segments (probably sacral), as "recurrent parasympathetic" fibers. The work of Johnson (15) in the sacral sympathetic chain of the cat does not give any anatomical support for such a distribution.

The anatomical evidence at the present time does not support a direct

innervation of skeletal muscle by the sympathetic nervous system (3, 16, 17, 18, 19, 20, 21, 22, 23). The observations recorded here still require an indirect action upon the muscle (see Gasser, 7). Cannon and Bacq (24) report that a substance "Sympathin" is elaborated at the endings of the thoracolumbar sympathetic fibers. This substance is thought to resemble adrenalin. It is debatable whether all of the postganglionic sympathetic fibers elaborate the same substance. The recent work of Burn (25) shows the presence of vasodilator responses from sympathetic trunk stimulation. He recognizes the possibility that stimulating the same nerve fiber might either produce vasodilatation or vasoconstriction according to the conditions of the stimulation but some of his results can be explained only on the assumption that there are two sets of fibers, one for vasoconstriction and one for vasodilatation.

The nervous pathway for vasodilatation to skeletal muscle is not known definitely. Bayliss (26) still believed that the muscles received vasodilator impulses from the dorsal roots due to the fact that there was a very small vasodilatation in the leg following removal of the skin. Langley (27) criticized this work of Bayliss on account of the fact that only one experiment was made, that removal of the skin would probably interfere with the circulation of the muscle and that there would be other tissues, in addition to skeletal muscle, left following removal of the skin, in which dilatation might be present. Hartman, Evans and Walker (28) observed directly in the sartorius muscle of the cat that stimulation of the sympathetic chain in the lumbar region by rapidly repeated induction shocks produced only dilatation of the capillaries and venules. They did not see vasoconstriction with any rate or intensity of stimulation they used. It may very well be that the vasodilator responses from dorsal root stimulation are limited to the skin and subjacent tissues and that the muscles receive vasodilator impulses from the sympathetic pathways. This vasodilatation may be produced by a "vasodilator substance" which in normal physiological activity is limited in its action to the blood vessels. With the type of stimulation required by the Sherrington phenomenon, it may be produced in such concentration that it "leaks out" or overflows by way of the tissue fluid to the surrounding sensitized skeletal muscle fibers.

These observations place the Sherrington phenomenon in a similar category with that of Rogowicz (29). Following section of the facial nerve, he found that stimulation of the cervical sympathetic produced, in addition to flushing and vasodilatation in the gums and lips, a contracture of the upper lip. This has been confirmed by von Euler and Gaddum (30). The one discrepancy however is that they were able to produce the contracture by stimulation of the preganglionic fibers while we have been unable to do so in the muscles of the hind limb. We have obtained it only on stimulation of the postganglionic sympathetic fibers.

In 1928 we (11) stated, "It should be remembered that some nerves act indirectly, sending chemical substances to bring about a response rather than carrying the impulse to the tissue cells." We are in agreement with Cobb and Wolff (28) who state, "It is probable that sympathetic nerves do not end in striated muscle, but that stimulation of the sympathetic nerves to the vessels of a muscle may alter the tissue fluid or blood in such a way as to change the muscular contractility. For example, sympathetic stimulation enables skeletal muscle, especially with the onset of fatigue, to contract for a longer period and more forcibly." We consider the Sherrington phenomenon with the other pseudomotor contractures as physiological artefacts. They do not represent normal functional mechanisms but rather muscles sensitized by section of their motor nerves to chemical substances, whatever they may be, produced under the influence of nerve fibers.

It is a pleasure to acknowledge the counsel and constructive criticism which we have received throughout the progress of this work from Dr. Herbert S. Gasser of the Cornell Medical School.

CONCLUSIONS

1. The Sherrington phenomenon in the quadriceps and gastrocnemius muscles is due to nerve impulses and not to spread or non-nervous conduction.

2. It is not produced by conduction over somatic motor nerves coursing in an atypical manner.

3. Antidromic conduction over somatic afferent fibers does not give an adequate explanation.

4. It is produced by strong, rapid faradic stimulation of post-ganglionic sympathetic fibers.

5. The possibilities of a humoral mechanism are discussed.

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STUDIES IN THE NORMAL HUMAN WHITE BLOOD CELL PICTURE

I. Variations in Recumbent Basal Subjects and in Individuals with Change of Posture

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Distinct variations in the number of the white blood cells in the peripheral circulation have been demonstrated by various investigators (Sabin, Cunningham, Doan and Kindwall, 1925; Shaw, 1927; Medlar, 1929; Smith and McDowell, 1929, and Soeriobroto, 1930). Garrey (1929) emphasized the fact that the variations were due to the subject not being kept in an undisturbed state. Ponder, Saslow and Schweizer (1931) explained some of the fluctuations on the basis of the error inherent in the technique. Due to the discrepancies in the results of the above investigators it has seemed advisable to us to repeat this work. We have used a series of normal subjects and have given full considerations in particular to the points stressed by Garrey and Ponder, Saslow and Schweizer. There have been widespread differences in the literature as to the effect of posture, also (Ellermann and Erlandsen, 1911; Hasselbalch and Heyerdahl, 1908; Jacobsen, 1926; Jorgensen, 1917; Garrey, 1929, and Garrey and Butler, 1929). We have attempted to settle this problem by a series of investigations on both normal and abnormal subjects.

Methods. I. *Recumbent basal subjects and individuals with mild activity.* Sixteen male adults were used. Thirteen of these individuals were in excellent health. Two developed acute coryzas a few days later and another had been exposed, at his home, to repeated respiratory infections and for several days had had some trouble with ease of exhaustion. Each of these individuals came into the laboratory in the morning without food and rested in a recumbent position for one hour before having any samples of blood taken. In no instance was food given until the period of observation was over, but small amounts of water were given when the subject desired it. Ten of the individuals remained recumbent during the entire

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period of observation. Six of these ten subjects were under observation from 9:00 a.m. to 1:00 p.m. and the remaining four from 8:00 a.m. to 1:00 p.m. The six additional subjects were allowed to be up and around the laboratory with mild exercise for a short period (1 to $1\frac{3}{4}$ hr.) during the four hour period of observation from 9:00 a.m. to 1:00 p.m. The following observations were made at 15 minute intervals during the period of the experiment in 12 of the subjects:

a. Determinations of the total number of the white blood cells on the basis of samples collected in two Thoma pipettes. The number of white blood cells found in a total of 8 sq. mm. (4 sq. mm. in each of two drops) was determined for each pipette after thorough shaking in an automatic shaker. In other words, the total white blood cell counts were based on the number of cells found in 16 sq. mm. (8 sq. mm. for each pipette) on Levy-Hauser counting chambers. These counts were made by one or the other of two observers, whose technical error had been determined by means of repeated counts on the same sample of oxalated blood.

b. Schilling differential white blood cell counts of 200 cells on fixed smears stained with Wright's stain. In making these differential counts, 100 cells were counted on each of two cover glasses.

c. Simultaneous supravital neutral red differential white blood cell counts of 100 cells each by two observers.

d. Determinations of the number of white blood cells in the saliva. These observations will be made the subject of a separate communication.

The same observer carried out the same procedures in all of the experiments except that the determinations of the total number of the cells were made in part of the subjects by one observer and in the remainder by another. This change was made in order to exclude the personal equation as far as possible. The same two pipettes were used throughout the work except when breakage made this impossible. All pipettes were Thoma white blood cell diluting pipettes with Bureau of Standards certificates. Levy-Hausser counting chambers with Neubauer ruling and Bureau of Standards certificates were used.

In four of the subjects the same procedures as above were carried out except that the differential counts were omitted.

II. *Individuals subjected to change of posture.* Our observations under this heading may be conveniently divided into three groups. The first group comprises 31 normal men between the ages of 20 and 40 years. These individuals were on the resident staff of the Strong Memorial and Rochester Municipal Hospitals. In each instance the first sample of blood was taken from the finger before the subject arose in the morning, after a good night's rest. (There was one exception in this group. The first sample from subject 1 was obtained after he had been recumbent for 30 minutes without breakfast.) Then, the subject stood erect and a second

sample was taken immediately. A third sample was taken five minutes after assuming the erect posture in all but one of these subjects. After thorough shaking in an automatic shaking apparatus a drop of diluted blood from each of the three pipettes was placed in a Levy-Hausser counting chamber with Neubauer ruling. The cells in each of four square millimeters were counted for each drop. The counts were made by two different observers in order to make sure that the personal equation was not entering into the results. Bureau of Standards counting chambers were used in all of this work. Thoma white blood cell diluting pipettes with Bureau of Standards certificates were used in all but 9 instances.

The second group is composed of four normal young adults. These individuals came to the laboratory at 8:00 a.m. without breakfast and following a good night's rest. They lay down on a bed for an hour, at the end of which time a lobe of one ear was punctured and blood obtained in each of two white blood cell pipettes. Then, the subject assumed the erect posture and immediately thereafter blood was obtained in two white blood cell pipettes. Five minutes after standing, blood was obtained in two other white blood cell pipettes. After thorough shaking, diluted blood from each pipette was placed under each of two sides of a Levy Hausser counting chamber with Neubauer ruling. The cells in a 4 sq. mm. area were counted for each drop. In other words, the cells in 8 sq. mm. were determined for each pipette. After the first three sets of samples of blood were obtained, the subject was given a large breakfast consisting of: orange sections, 100 grams, sugar, 5 grams, bread, 50 grams, butter, 20 grams, potato, 150 grams, milk, 20 cc., tenderloin steak, 240 grams, water (room temperature) as desired up to 500 cc. Immediately upon finishing breakfast the subject again assumed the recumbent posture. One hour later, the same series of white blood cell counts was made as prior to breakfast. A third series of counts was made after the subject had been recumbent another hour and a fourth series after he had assumed the recumbent posture for still another hour.

The third group is composed of 18 individuals representing a variety of clinical conditions. The same technique was used in all of the subjects in this group as in the first group.

RESULTS. I. *Recumbent basal subjects and individuals with mild activity.* The variations in the total number of the white blood cells of four different basal recumbent individuals are shown in chart 1. The curves for five of the other individuals in this group were of the same nature as those shown. The one remaining subject (A. J.) in this group showed much greater fluctuations in the total number of white blood cells. This individual developed an acute coryza a few days later. Whether this influenced his white blood cell picture cannot be stated. However, the findings in his case were so different from those of the 9 others in the group that we felt

justified in classifying him as an exception to the rule. It is obvious that the variations in all of the subjects, with this one exception, are of a minor degree.

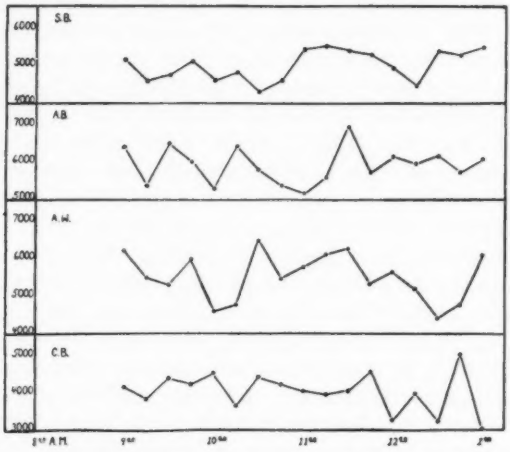

Chart 1. Curves representing the total number of white blood cells determined at 15 minute intervals in four basal recumbent subjects.

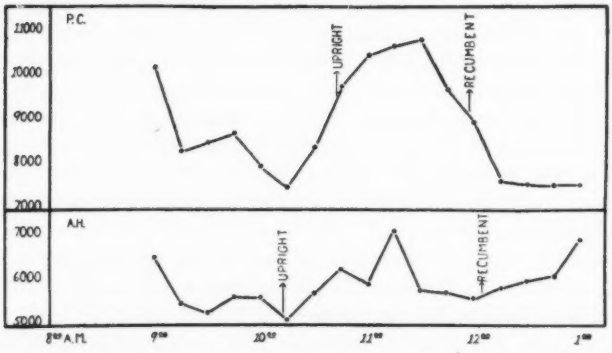

Chart 2. Curves representing the total number of white blood cells determined at 15 minute intervals in two subjects allowed slight activity. *A. H.* represents the typical curve. *P. C.* represents the findings noted in one individual with a mild respiratory infection.

The curves for the total number of the white blood cells for two of the individuals who were allowed short periods of activity are given in chart 2. The curves for four of these subjects differed in no essential way from those of the 9 subjects who were kept recumbent. A. H. in chart 2 is

typical of these four. The other two subjects showed deviations almost three times as great as our range of error. One of these subjects (P. C.), who showed wide variations, developed an acute coryza 2 days later. The other subject (H. P.) stated that acute respiratory infections were present in his family. He did not have an acute coryza but had for some days been easily exhausted.

The range of variations in percentages of cells represented in a total count is shown in table 1 for supravital and fixed preparations. The striking

TABLE 1

Range of variations of different white blood cells in percentages in supravital and fixed preparations

BASAL SUBJECTS 4 HOUR PERIODS		NEUTROPHILS	NON-MOTILES	EOSINOPHILS	BASOPHILS	LYMPHOCYTES	MONOCYTES	DEGENERATED CELLS
S. B.	Supravital	58.5–69	0–7.5	1–7.5	0–1.5	19.5–27.0	2–8	0–4.0
	Fixed	47.0–57		1.5–5	0–1.5	32.0–42.0	3.5–9.5	0–2.5
A. B.	Supravital	56.5–72.5	0–9	2–6	0–1.5	13.5–27	3.0–13.5	0–2.0
	Fixed	54.0–67.0		1–6	0–1.0	19.5–31	4–11	0–3.0
C. B.	Supravital	50–62.5	0–6	2–8.5	0–2	23–39.5	2.5–9	0.5–4.0
	Fixed	43.0–57.0		1.5–4.5	0–2	31–45	5–10	1–5
J. G.	Supravital	42.5–58	0–3	2–6.5	0–2	30.5–41	3.5–10.5	0–2
	Fixed	40.5–51		1–4	0–1	35.5–51	4.5–10	0–2.5
J. J.	Supravital	50–69	0–4	1.5–5.5	0.5–4.5	19.5–34.5	5–10.5	0–3
	Fixed	44.5–55		0.5–4.5	0–1.5	33.5–41	4.5–11.5	0–4.5
A. W.	Supravital	65–75	0–6	0.5–5	0–3.5	14.5–27.5	3–8.5	0–2.5
	Fixed	58–71		0–3.5	0–1.5	19.5–30	3.5–9.5	0–3
Average supravital		53.7–68.0	0–5.9	1.5–6.5	0.1–2.5	20.1–32.8	3.2–10.0	0.1–2.9
Average fixed		47.8–59.7		0.9–4.6	0–1.4	28.5–40.0	4.2–10.2	0.2–3.4

findings in this connection were the diminished percentages of neutrophils and eosinophils and the increased percentage of lymphocytes in fixed preparations. These were constant findings in six basal recumbent subjects and in the six subjects allowed short periods of activity. In general, the curves representing the absolute number of each of the groups of cells had the same configuration as that for the total number of cells. In as much as the greatest percentage of cells was neutrophils, the curves for these cells more closely paralleled those for the total number of the cells.

The percentage of non-motile cells was constantly low, 9.0 per cent being the highest figure obtained. It was noted that the two observers practi-

cally never found them at the same intervals, and further, that the preparation in which they were found was the thinner of the two.

No rhythm could be detected in any of the curves for the total number of the white blood cells or for the absolute number of any of the various cells constituting the total number.

TABLE 2

Statistical analysis of the results obtained by doing total white blood cell counts on: a, single specimens of ozalated blood; b, a group of basal recumbent subjects; c, a group of subjects with mild activity; and d, three individuals with mild infections

SUBJECT	NUMBER OF DETERMINATIONS	ARITHMETIC MEAN	MEAN DEVIATION	STANDARD DEVIATION	PROBABLE ERROR	COEFFICIENT OF VARIATION	
						per cent	
Single specimen	15	6333	±283	±342	±231	±5.4	Observer A
Single specimen	15	6661	±289	±335	±236	±5.0	Observer B
S. B.	17	4995	±335	±392	±264	±7.8	
C. B.	17	4039	±387	±506	±341	±12.5	
J. G.	17	4750	±374	±471	±218	±9.9	
A. B.	17	5917	±397	±477	±324	±8.1	
A. W.	17	5533	±513	±609	±411	±11.0	"Basal"
J. J.	17	5964	±552	±656	±442	±11.0	
S. L.	21	6000	±492	±616	±416	±10.3	
J. R.	21	5146	±699	±824	±558	±16.0	
J. W.	21	5642	±382	±495	±328	±8.8	
Mean	9	5332	±461	±561	±380	±10.6	
A. H.	17	5910	±396	±512	±346	±8.6	
O. M.	17	7238	±452	±544	±365	±7.5	
R. L.	17	5949	±502	±607	±407	±10.2	"Activity"
F. D.	17	6398	±563	±716	±480	±11.2	
Mean	4	6364	±478	±595	±399	±9.4	
A. J.	21	5916	±1465	±1704	±1150	±28.8	Mild infection
P. C.	17	7850	±1031	±1125	±753	±14.3	Mild infection
H. P.	17	9350	±1213	±1296	±868	±13.9	Mild infection

Table 2 gives results of the above experiments in mathematical form. It also gives for comparison the results obtained by two observers in making total counts at repeated intervals on the same sample of blood.

II. *Individuals subjected to change of posture.* The average results obtained in the individuals of group I are shown in table 3. It will be seen that the average number of white blood cells for the entire group in the recumbent position was 6280 per cu. mm. as against 5597 per cu. mm. immediately upon standing and 6270 per cu. mm. 5 minutes after assuming

the erect position. The differences between counts for each individual were small. The counts made immediately after assumption of the standing position revealed only 2 instances when the differences amounted to 2000 or more cells whereas those made at the 5 minute period revealed only 4 instances when differences of this magnitude were found. Most of the variations were well within the limits of error inherent in the technique. They were distinctly less than the variations which have been shown to occur in normal basal individuals over a period of four hours when white blood cell counts have been made at 15 minute intervals. Further analysis shows that the counts made immediately after standing showed an increase in 5 instances, a decrease in 24 instances and no change in 2 instances. At the 5 minute interval there was an increase in 16 and a decrease in 14 of the subjects.

The average results in the second group are shown in the same table. The variations were of approximately the same magnitude as those in the

TABLE 3

Average results obtained in 65 observations on change of posture

GROUP	RECUMBENT	UPRIGHT*	UPRIGHT†	REMARKS
I	6280	5597	6270	Average results in 31 normal subjects
II	5707	5245	5212	Average of 16 sets of observations on 4 normal subjects
III	6869	6461	7063	Average results in 18 abnormal subjects

* Immediately after assuming erect posture.

† Five minutes after assuming erect posture.

subjects of group I. The average number of white blood cells in the recumbent posture was 5707 per cu. mm. as against 5245 per cu. mm. immediately after standing and 5212 per cu. mm. 5 minutes after assuming the erect posture. The variations immediately after standing were above 2000 per cu. mm. in only 2 instances and at the 5 minute interval there were no differences of as much as 2000 per cu. mm. There were 4 instances when the counts were increased and 12 when they were decreased immediately after standing. The same figures hold for the 5 minute period. These variations were not constant for the different individuals, decreases and increases being found in the same individual at different times. Bureau of Standards pipettes and counting chambers were used in all of the experiments in this group.

Table 3 shows also the average results obtained in the individuals of group III. It will be seen that the results are entirely comparable with those obtained in the other two groups. The average number of white blood cells per cu. mm. for this group when recumbent was 6869 as against 6461 immediately after standing and 7063 5 minutes after standing.

The total white blood cell count was increased in 5 instances and decreased in 13 instances immediately after standing whereas at the 5 minute interval it was increased in 8 instances and decreased in 7 instances.

DISCUSSION. We have taken into careful consideration the variations which may occur as a result of errors in technique as emphasized by Ponder, Saslow and Schweizer (1931). The results shown in table 2 indicate that we obtained errors of approximately the same magnitude as these investigators. However, the variations in the total number of the white blood cells in the case of the individual subjects were slightly greater than those found when repeated counts were made on the same specimen of blood. Hence it has seemed to us that at least a part of the small fluctuations observed in the curves of the subjects in the basal and activity groups was due to physiological variations. Similar variations were found in the numbers of the different types of white blood cells making up the total.

Of course, another great source of error was introduced in the differential counts, namely, the distribution of the cells in the preparations in which the cells are counted. Barnett (1933) has emphasized this unavoidable error. Our results have agreed with his statements in this connection.

We have been unable to find any evidence of periodicity in the total number of the cells or in the number of any of the constituents making up the total. In this we are in agreement with Ponder, Saslow and Schweizer (1931), Garrey (1929) and Shaw (1927).

Sabin, Cunningham, Doan and Kindwall (1925) emphasized the non-motile cell as a possible indication of one way in which neutrophils degenerated. They attempted to correlate their appearance in "showers" with diminution in the total number of neutrophils. Smith and McDowell (1929) inclined to the belief that most of the non-motile cells were artefacts. We agree with these observers. This assertion is based upon the following facts: 1, in only rare instances did we find an appreciable number of these cells; 2, when they were found by one observer, they usually were not found by the other observer in a preparation taken at the same time; and 3, they tended to occur only in preparations that were too thin.

Schilling differential counts on the individuals in the basal and activity groups failed to reveal any fluctuations in the "stab" forms indicating that the physiologic variations which did occur were probably not due to changes in activity on the part of the bone marrow.

Our results tend to indicate that short periods of mild activity in normal subjects may not be associated with any corresponding changes in the total number of the white blood cells. Whether the marked variations which did occur in two of our subjects when mild activity was allowed were due to mild infections or to the exercise cannot be stated.

The results obtained in the 65 observations on change of posture clearly indicate that this factor influences to no appreciable degree the total num-

ber of white blood cells in the peripheral blood. Certainly the number of cells is not increased by changing from the recumbent to the erect posture. Since there was a preponderance of instances (49 out of 65) in which the total number of white blood cells was diminished immediately upon standing, it may be that there is a temporary diminution immediately upon standing.

CONCLUSIONS

1. Under basal conditions, the total number of white blood cells in normal subjects shows only slight variations.

2. Similar findings have been obtained in normal subjects who have been allowed short periods of mild activity.

3. No rhythm is demonstrable for the total number of the white blood cells or for the absolute number of any of their constituents.

4. Non-motile cells are probably artefacts, *under the conditions of the present experiments.*

5. The slight fluctuations in the total number of the white blood cells which do occur are probably not the result of variations in bone marrow activity.

6. Change of position has not been found to be associated with any significant variations in the total number of the white blood cells in the peripheral blood.

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STUDIES ON THYROGLOBULIN

III. The Thyroglobulin Content of the Thyroid Gland

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Tamback (1) in 1898 observed that about 96 per cent of the iodine in thyroid glands could be precipitated with alcohol, indicating that most of the iodine was present as thyroglobulin. Since that time several investigators have presented indirect evidence pointing in the same direction. Recently Lawson (2) found only a small percentage of inorganic iodine, confirming previous workers. Harington and Randall (3) have presented further evidence that most of the iodine is in protein combination. About a year ago we became interested in quantitative measurements of thyroglobulin in thyroid glands and worked out a rapid method for the estimation. Since thyroxine and diiodotyrosine have been isolated from the glands (4), it seems essential to know more about the quantitative aspects of the iodine compounds.

Methods. Fresh thyroids were finely ground and repeatedly extracted with 0.1 molar sodium acetate using 1 cc. per gram of glands. The 24-hour extract was squeezed through a cloth and fresh sodium acetate added. (Physiological saline works as well for extraction.) From 6 to 10 such extractions remove practically all (about 97 per cent) of the iodine which confirms Tamback's results. An attempt was made to precipitate the thyroglobulin, measuring the iodine in the original fluid and in the precipitate. Isoelectric precipitation or precipitating agents requiring an acid medium are objectionable since thyroxine is insoluble in acid. Since Tamback had employed alcohol, this was tried with good success under carefully controlled conditions. It was found that adding three volumes of 95 per cent ethyl alcohol to the thyroid extract completely precipitated the thyroglobulin within two hours. It was essential for the extract to be neutral in reaction. The precipitations were carried out in conical centrifuge tubes and after standing for two hours were centrifuged at high speed. The supernatant fluid was decanted and the tubes drained for a few minutes. The precipitate was transferred to a nickel crucible for iodine estimation. The small amount of fluid adherent to the precipitate

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was found to be insignificant. Controls were run by adding known quantities of KI, methylene iodine, thyroxine and both the acid-soluble and the acid-insoluble fractions from pepsin-digested thyroglobulin. This method has been employed for the estimation of thyroglobulin in samples of dog thyroids collected in the laboratory and in hog thyroids some of which came from Chicago, some from Fort Worth, Texas, and some from West Fargo, North Dakota.[2]

RESULTS. In testing the thyroid extracts to see if other forms of iodine would precipitate, the quantity of substance added depended upon its iodine content. An amount of KI was added which had an iodine content equal to the total iodine content of the thyroid extract but the quantity of iodine in the alcohol precipitate was not influenced. A similar quantity of digested thyroglobulin or ten times that quantity of thyroxine likewise had no effect. It would appear that under the given conditions, only thyroglobulin or closely related heavy molecules are precipitated.

TABLE 1

Showing the percentage of total iodine precipitated from fresh thyroid extracts by the addition of alcohol

SPECIES	SOURCE	I_2/GM. GLAND	PER CENT I_2 AS THYROGLOBULIN
Dog	Laboratory	0.526	93 6
Dog	Laboratory	0.836	96 6
Hog	Central States	0.977	97.7
Hog	Central States		100.0
Hog	Texas	1.477 mgm.	96.4
Hog	Texas	0.987	98 08
Hog	N. Dakota	0.593	100 0
Hog	N. Dakota	0.386	100 0

When the above method was applied to fresh thyroid extracts containing practically all of the iodine in the glands, it was found that *most* of the iodine was present as thyroglobulin.

In table 1 the lowest and highest values from each group are recorded. Five samples of each have been analyzed but the intermediate values are omitted to conserve space.

DISCUSSION. Fenger, Andrew and Vollersten (5) have shown that thyroids collected at Fargo have about one-half as much iodine as those collected at Fort Worth. It can be seen in table 1 that in either case nearly all of the iodine is in thyroglobulin combination. It must be borne in mind that the iodine methods are not perfect and an observation of 100 per cent of thyroglobulin does not exclude the presence of small quantities of iodine either in organic or intermediate forms.

[2] We are indebted to Dr. F. Fenger and to Mr. Andrew of Armour & Co. for fresh thyroids.

Since over 95 per cent of the iodine was found as thyroglobulin, we have not investigated the nature of the remaining small fraction. No doubt all stages of combination would be represented from inorganic iodine to the ultimate form, thyroglobulin. It seems significant that such a large proportion is present as the protein especially since thyroglobulin is secreted into the blood and lymph (6), (7). The acid-soluble and the acid-insoluble fractions which Kendall describes after hydrolysis of thyroids must be looked upon as decomposition products of thyroglobulin. In view of our present knowledge, the question arises, what is the thyroid hormone? If we define a hormone as being produced, stored, and secreted by a gland certainly thyroglobulin fits the definition. Apparently thyroxine is not present in the gland in appreciable quantities and we are not acquainted with any physiological evidence of the secretion of thyroxine as such. At the present time it seems more desirable to us to look upon thyroglobulin as the hormone instead of thyroxine as many textbooks have done.

SUMMARY

1. Using a method which distinguishes between thyroglobulin and other forms of iodine, it has been found that most of the iodine in thyroids from hogs and dogs is present as thyroglobulin.

2. The percentage of thyroglobulin was the same in samples of hog thyroids collected in different regions of the country even though the iodine content was quite different.

3. The question of the thyroid hormone is discussed.

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IS THERE A SPECIFIC DIURETIC HORMONE IN THE ANTERIOR PITUITARY?

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Teel (1) in 1929 reported marked diuresis in dogs receiving injections of growth hormone. Some preparations were apparently free from the diuretic principle yet contained the growth hormone. His preparations were made by neutralizing alkaline extracts from fresh anterior lobes. We have produced diuresis with an acid extract similar to his observations and have a plausible explanation for it.

METHODS. We have used two extracts, one prepared from dried anterior lobes by Loeb's method for the thyroid stimulating principle and the other by a much similar method employing fresh glands. Both of these methods involve acid extraction of the glands followed by neutralization. In most of our studies we have injected subcutaneously only 10 cc. of these extracts which is equivalent to 0.5 gram of dried powder daily or about 2 grams of fresh anterior lobe. A total of 24 dogs has been injected, 18 normals and 6 completely thyroparathyroidectomized. The maximum time of injection was 12 days.

RESULTS. In all of 18 normal dogs a marked diuresis was observed similar to that described by Teel. There is usually no effect the first 24 hours after starting the injections but on the second day both water intake and urine volume increase. The maximum is reached after 3 to 5 days and polyuria continues for several days. In one animal injected for 12 days, the urine output returned to the control level the last two days of the injection. After cessation of injection the urine volume gradually comes back to normal within 4 to 5 days. The water intake closely parallels the urine output. The amount of the diuresis varies in different dogs, being only two or three times the control level in a few dogs, most animals showing an increase of five times, while a few increased their volume by as much as ten times the control period.

No diuresis was observed in a thyroidectomized dog when injected with the same extracts which were effective in normal dogs. Likewise there was

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no increase in thirst nor rise in metabolism. This animal had been thyroidectomized over a year previously. The same results have been obtained in 5 other dogs recently thyroidectomized and maintained free from tetany with calcium gluconate. In these animals the diuresis could be produced by feeding them thyroid and although the thyroid therapy might be stopped during the injection of the anterior lobe extracts, the urine output returned to normal. Two typical cases are shown in the accompanying graph, one animal being normal while the other was thyroidectomized.

Discussion. The fact remains that Teel used alkaline extracts of the anterior lobe while we have used acid extracts. Therefore, it is possible

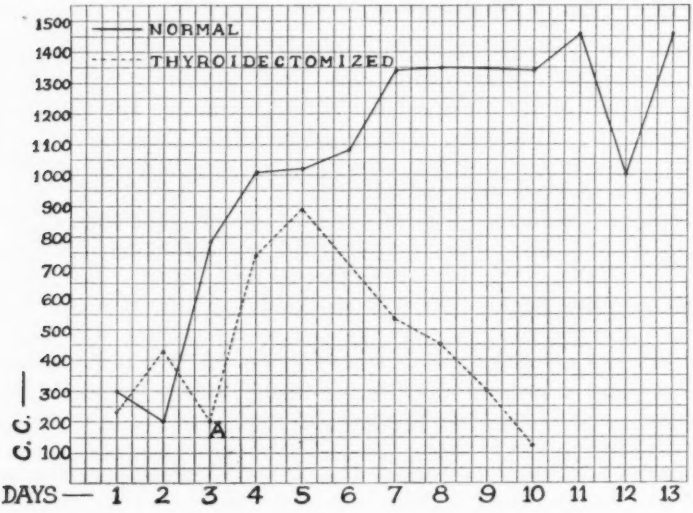

Fig. 1. Showing the urine output of a normal and a thyroidectomized dog during the injection of anterior pituitary extract. At point *A*, a single dose of thyroid extract (14.2 mgm. iodine) was administered to the thyroidectomized dog.

that the diuretic factor is different in the two series of experiments. However, Loeb and Bassett (2) have shown that the thyroid stimulating factor may be obtained by alkaline extraction. At any rate the observations are similar and in our experiments the diuresis has parallelled the basal metabolism. Our experiments do not allow us to conclude that Teel's observations were due to stimulation of the thyroid but they do suggest such a mechanism. Until further evidence appears for a specific diuretic hormone, it seems better to have one less postulated principle in an already over-crowded field.

SUMMARY

Marked diuresis which occurs in normal animals following the injection of extracts from anterior bovine pituitaries was not observed in thyroidectomized dogs. It is suggested that the diuresis is a result of thyroid stimulation.

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PULMONARY ARTERY PRESSURES

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Talma (1881), Bradford and Dean (1894), Knoll (1888) and Plumier (1904) have studied the pressures in the pulmonary arteries in animals and found systolic and diastolic variations due to respiration.

Wiggers (1910) made his first report upon the maximal and minimal pressures found in the pulmonary arteries of dogs. He showed that the mean pressure averaged 18 mm. Hg. Pressures taken with a Hürthle manometer gave an average systolic pressure of 31.1 mm. Hg and 12.3 mm. Hg diastolic pressure on inspiration and 36 mm. Hg systolic pressure and 17.1 mm. Hg diastolic pressure on expiration. In 1912, Wiggers reported the results of additional work in which a sensitive membrane manometer was used. This work was done in the closed chest. The maximal pressure averaged 43.5 mm. Hg and the minimal 11.9 mm. Hg. Wiggers (1914) reported additional work which had been done by using a special new manometer. The pressure curves were recorded by the optical method.

In a series of experimental investigations on the pulmonary circulation on dogs the authors observed (9, 10, 11, 12) the effect of respiratory variations on the pulmonary artery pressure although actual pressure determinations were not made. This report deals with experiments carried out to determine pulmonary artery pressures in the closed chests of dogs where a new method of approach has been used.

METHODS. We have studied the pressures in the pulmonary artery in the closed chest by exposing this vessel through an opening made in the mediastinum. The method of approach has been described in detail by one of us (C. F. H.).[1] An incision was made over the trachea in the midline of the neck. Through this incision the dissection was extended into the thorax between the two pleural cavities until the pulmonary artery was exposed. Pressures were taken with a micro mercury-manometer, to which a needle (15 gauge) was connected by a thick walled rubber tube. The needle was curved at a right angle about 2 cm. from the point. The needle in each experiment was thrust into the artery with the mouth of the needle

[1] Since the original publication, the author has found that Terry has used this method. See reference in bibliography.

directed against the direction of flow. The pressure changes in the manometer were recorded on a smoked drum by using a thin aluminum writing point attached to a rod which was suspended on a hard rubber float with a concave surface. The bore of the manometer was 2 mm. In these experiments the pressure within the pleural cavities was not disturbed. Five experiments were done under ether anesthesia. Respirations were recorded on the drum through a valve in the tracheal cannula. Respirations and second intervals were recorded simultaneously with the pulmonary artery pressure. In each experiment, after the vessel was exposed, the needle and tubing were filled with 10 per cent sodium citrate solution and held on an exact level with the pulmonary artery. The level of the top of the column of mercury was placed on the same level as the needle when

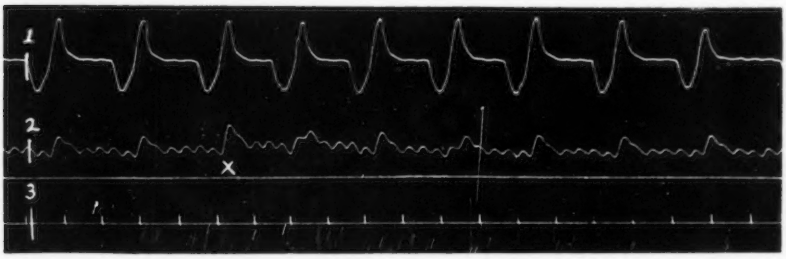

Fig. 1. Pulmonary arterial pressure tracing. Corresponding points on respiration *1*, pulmonary artery pressure *2*, and timing interval *3* are designated by perpendicular lines immediately below the figures *1*, *2* and *3*. The base line is the line immediately below the pulmonary artery pressure tracing. Time interval in one second. The reproduction shows the respiratory variations produced on the pulmonary systolic and diastolic curves. At the point *X* there is a direct superimposition of ventricular systole on expiration.

thrust into the vessel. We were careful to hold the mouth of the needle directly against the flow of blood so that we should be measuring head pressure.

Five experiments were done in which we measured only the maximal pressure by making direct readings from a water manometer. In these experiments a simple piece of right angle glass tubing was used. The curved needle was attached to the tube with a short piece of rubber tubing. The needle and one arm of the glass tubing were placed on the same level with the artery and the column of water was allowed to seek its level. The rubber tube was then clamped and the manometer was filled with water to a point which we thought might be the point of approximate pressure. The needle was then thrust into the vessel with the mouth of the needle directed against the head pressure or flow and the clamp on the rubber

tubing was released. The column of water was allowed to adjust itself and readings were made at the adjusted maximal point. Variations in oscillation of the volume of water could be seen with each respiratory cycle but we made no attempt to make readings which we might have attributed to the different phases of respiration. Ether was used for anesthesia in the first group of experiments and dial[2] with urethane (0.6 cc. per kilo) was given in the second group. Pressure readings in both groups of experiments were made while the animals were under light anesthesia.

RESULTS. The maximal pressure averaged 16 mm. Hg in the first group of experiments and averaged 220 mm. of water or 16 mm. Hg in the second group of experiments where direct readings were made. Figure 1 shows the typical curves we obtained. A number of curves in each experiment were measured to get an average of pressures in expiration and inspiration. Table 1 gives the average pressures recorded in each experiment. Average

TABLE 1

Pulmonary arterial pressures expressed in millimeters Hg

EXPERIMENT	EXPIRATION		INSPIRATION	
	Systolic	Diastolic	Systolic	Diastolic
1	14	6	8	2
2	16	10	12	8
3	18	14	12	8
4	16	10	8	4
5	16	12	10	8
Average.....	16	10.2	10	6

systolic pressure of 16 mm. Hg on expiration; and systolic pressure of 10 mm. Hg and diastolic pressure of 6 mm. Hg was obtained on inspiration. The maximal pressures in our experiments were much lower than the pressures reported by Wiggers who used the optical method. We believe that the maximal pressure records obtained in these experiments indicate very closely the true pressures in the pulmonary artery of the dog. It has been shown on the smoked drum tracings that the mercury has followed the changes in pressure caused by respiration, i.e., decrease in systolic and diastolic pressure during inspiration and increase in systolic and diastolic pressure during expiration, as has been shown by Knoll and Wiggers. According to Wiggers a third beat of the ventricle occurring during the phase of inspiration will be followed by a rise in pressure rather than a decrease. We were not able to show this.

We admit the mercury manometer will not record the finer details of the

[2] This anesthesia was kindly furnished for these experiments by the Ciba Company.

various pressure curves but we believe that this method has given us the true maximal pressures since the same results have been obtained by making direct readings with the simple water manometer.

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A STUDY OF SPONTANEOUS ACTIVITY IN THE EXCISED UTERUS OF THE RAT WITH PARTICULAR REFERENCE TO THE RÔLE OF THE OVARY AND INHERENT CHARACTERISTICS OF UTERINE MUSCLE

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In a previous paper one of us (Harne, 1931) described the spontaneous activity of excised uterine horns of sexually mature virgin rats, using an improved method. By this method we have studied the sexually immature uteri (using a very light lever) and also those of virgin animals in menopause, and after ovariectomy.

Sexually immature uteri of guinea pigs when treated by the method of Kehrer (1907) were found by Trendelenburg and Borgmann (1920) to be quiescent, an observation confirmed by Smith and McClosky (1924). In the rat we have no evidence that this condition obtains. On the contrary we have found rhythmic activity in all excised uteri used. Beginning with very young animals (3 days) we found rhythmic longitudinal contractions of about normal rate (3 contractions in 2 minutes). The curves were low with a broad oval peak (1, fig. 1). As the animals mature the curves increase in amplitude with some proportion to the increasing tissue mass, retaining, however, remarkable uniformity for the first 3 weeks (1, 2, 3, fig. 1), after which there is evidence that some mechanism, having power to alter tonus and type of contraction, has gained control (4, 5, 6, fig. 1). This irregularity can be followed throughout the remaining life of the animal, but exists to different extents, reaching a maximum during active sexual life (7, fig. 1). The question arises, does the ovary exert a dual influence over uterine activity, regulating one type and rate of contraction before sexual maturity and in menopause, and a different one during active sexual life? The influence of the immature ovary upon uterine activity is

Fig. 1. Showing the types of contractions obtained from excised uteri of sexually immature, mature, menopause and ovariectomized rats. 1, A; 2, B; 3, C; 4, D; 5, E; 6, F: left and right horns respectively of sexually immature animals. 7, curves from a series of animals in the different stages of the oestrous cycle. 8, left and right horn responses of an animal in menopause. 9, typical type of activity observed after ovariectomy.

Note: The amplification of the recording mechanism in curves 1, 2, 3, 4, 5, 6, and 8 was × 6. This does not hold for curves 7 and 9.

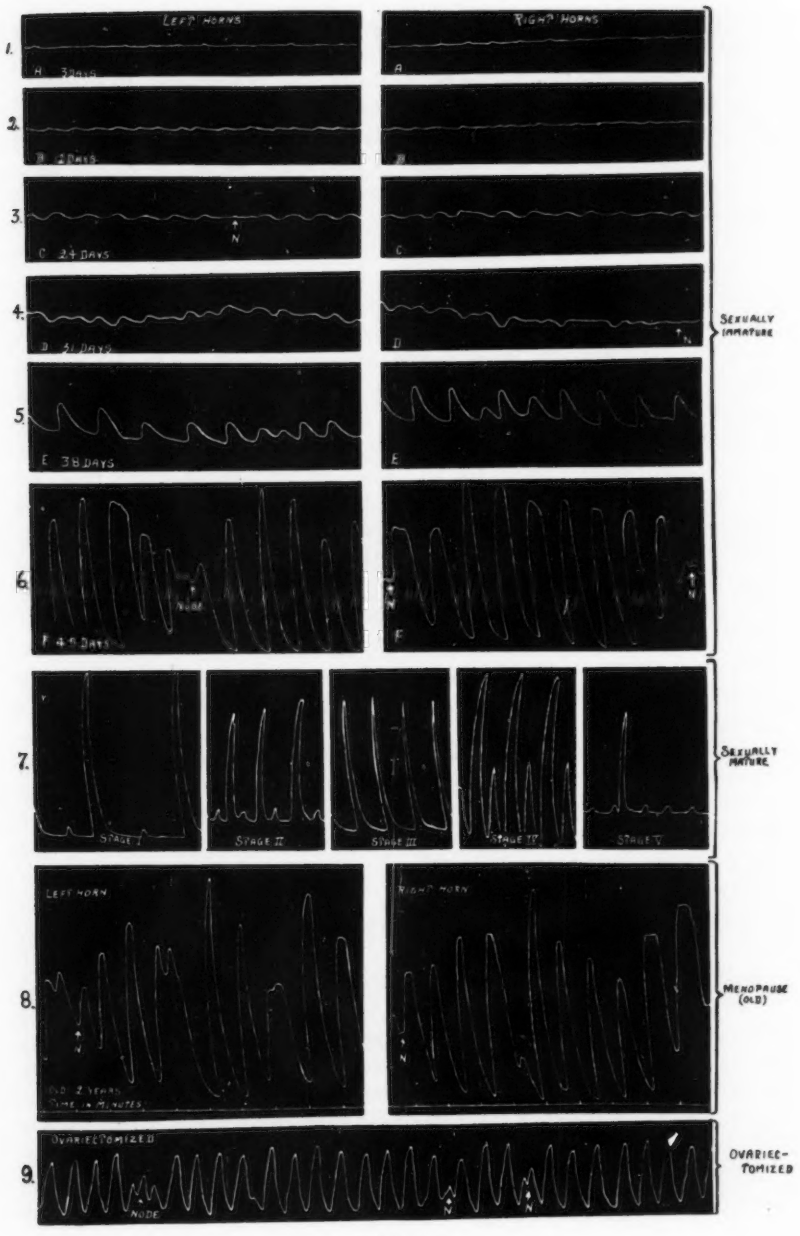
LEFT HORNS
RIGHT HORNS
1.
A 3 DAYS
A
2.
B 12 DAYS
B
3.
C 24 DAYS
C
4.
D 31 DAYS
D
SEXUALLY IMMATURE
5.
E 38 DAYS
E
6.
F 46 DAYS
F
7.
STAGE I
STAGE II
STAGE III
STAGE IV
STAGE V
SEXUALLY MATURE
8.
LEFT HORN
RIGHT HORN
O.D. 2 YEARS
TIME IN MINUTES
MENOPAUSE (OLD)
9.
OVARIECTOMIZED
NODE
OVARIECTOMIZED

Fig. 1

suggestive in view of recent work of Hohlweg and Dohrn (1932), but is not tenable in light of our experiments which will be discussed presently.

If a general rôle be ascribed to the ovary, two possibilities are presented. The extensive variation in rate and type of spontaneous activity associated with the oestrous cycle must be regulated by one ovarian substance with concomitant changes in the uterine histology, while the other, which persists from early life (about 21 days of age) as a definite type in the absence of cyclic changes of oestrus may be controlled by a separate substance or be due to the state of inherent activity of the tissue in the absence of the ovarian substance.

In this particular series of experiments we were concerned with the analysis of this condition and to this end have performed experiments upon immature and sexually inactive (old, menopause) animals to ascertain the type of contraction. It was found that the contraction types of animals immediately preceding active sexual life and during menopause closely resembled each other (6, 8, fig. 1), but were distinct from those found during active sexual life (7, fig. 1). Whether the absence of this type during sexual life is a result of it being overshadowed by the action of the ovarian substance cannot at this time be stated, but is suggestive upon the ground that during the oestrous cycle the tendency is to decrease activity over the period rather than increase it (Marrian and Newton, 1933). This forces the conclusion that the extensive variation in rate and type of spontaneous activity during the oestrous cycle is associated with active ovarian function, while the type which persists from early life is due to an indirect action of the ovary, if any, or an inherent characteristic of the tissue.

The effect of ovariectomy upon spontaneous activity of sexually mature animals. In light of the above experiments we have further checked these contraction types and the responsibility for their existence by a second series of experiments, assuming that control of uterine activity is exerted by the ovary during sexual life only, and that two distinct activities could not be mediated by a single substance. We have ovariectomized sexually mature animals and, at definite periods (10 to 50 days), excised the horns and tested their spontaneous activity. It was found that both the tonus and rhythm changes remained, but followed always a definite type, which is also observed in uteri immediately before sexual maturity and during menopause (6, 8, fig. 1). This contraction type is characterized by a grouping of maximal and submaximal contractions into a series of gradually changing types producing nodal points at intervals of from five to twelve minutes. The number of contractions in each group varies within narrow limits, but averages about ten.

From these experiments it appears that the cyclic changes alone of sexually mature animals are due to the ovary, and that the slightly irregu-

lar contractions and tonus variations of sexually immature, menopause, and ovariectomized animals are not mediated by the ovary, but are entirely distinct from the variations observed during the oestrous cycle. We believe that this contraction type is either an inherent characteristic of uteri under these conditions or one influenced by an anterior pituitary hormone. Presently we shall distinguish between these two possibilities.

The effect of theelin upon excised uteri. In this series it was thought that in view of recent work of Reynolds (1932), Kraus (1932), Severinghaus (1932), Hohlweg and Dohrn (1932) and others, the treatment of uteri of our experimental groups (immature, sexually mature, menopause, and ovariectomized) with theelin may produce a type of response that could be checked against one of the types observed under different conditions existing throughout life or under the conditions of our experiments. Reynolds (1932) reports upon a series of experiments in which the unanesthetized rabbit responded some hours after intravenous injections of theelin. The long latent period immediately suggests that the theelin has no direct effect upon smooth muscle of the uterus per se, but indirectly produces within the reproductive tract a condition which normally is accompanied by contraction of the circular muscle of the uterine wall. This point we have never disputed, but the action, if any, upon the longitudinal muscle is not definite and has not been satisfactorily explained.

We have determined contraction types upon animals of all our experimental groups using doses of theelin up to 50 rat units per horn, without any effect. However, in the excised tissue the experiments were terminated after three hours. In these experiments, there was no possibility of changing the characteristics of the tissue to that having normally a different rhythm as can be done in vivo. Therefore, if an immediate effect was not produced the experiment was declared negative.

With this experience we are convinced that any increased activity of uterine musculature after theelin must be an indirect one, and depends upon the establishment of conditions in the uterus which commonly show an increased activity of circular musculature. The long latent period observed by Reynolds (1932) and the passiveness of excised rat uteri to theelin are thus explained.

We believe definite contraction types are produced by equally definite tissue conditions, and the spontaneous activity of tissue of this series is representative of the tissue condition at the time of excision.

CONCLUSIONS

1. All excised uteri of this series respond spontaneously when treated by our method.

2. The sexually immature uteri may be divided into two groups: *a*, uteri of rats up to about 21 days of age which show a constant rate of contraction

with little change in tonus; *b*, uteri of rats from 21 days of age to sexual maturity which show a wave-like or nodal type of contraction which is similar to that of an animal in menopause.

3. Uteri from sexually mature animals (potentially active) show no contraction types comparable to those found in animals of the same series immediately before sexual maturity, menopause, or after ovariectomy.

4. Uteri from ovariectomized animals and those in menopause respond similarly to animals approaching sexual maturity.

5. The action of theelin was negative on all excised uteri of this series (sexually immature, mature, menopause, and ovariectomized).

6. The type of contraction observed in excised uteri is a result of the inherent characteristics of the tissue at the time of excision.

7. These experiments point to ovarian control of uterine activity during sexual life only, at which time its influence overshadows the nodal type of activity.

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NOTE ON CEREBROSPINAL ELASTICITY IN A CHIMPANZEE

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Opportunity was recently afforded to study the reactions of a chimpanzee (*Pan* spec. ?) to abrupt tilting from the horizontal to the two vertical positions (head-down and tail-down), with measurement of the pressure-changes and volume-changes in the cerebrospinal fluid. Because of the importance of this anthropoid, with its quasi-erect posture and its many close resemblances to man, it is considered desirable to record briefly the findings on the single animal.

The chimpanzee was a juvenile male, 40 pounds in weight, with a spinal length (occiput to last lumbar spine) of 328 mm. The animal was estimated to be five-and-a-half years of age, plus or minus one year. Although the animal had been used for previous studies on respiratory infections (common cold), it was in vigorous health at the time of experimentation. The technical procedures were carried out as in the previous observations on cats, dogs and macaques (1, 2, 4), with ether by intratracheal insufflation and with attachment of the animal to a tilting board. The pressure-changes and volume-changes in the cerebrospinal fluid on tilting were determined by the use of open-end manometers of different bores, connected with an occipital puncture-needle inserted into the cisterna magna. The differences in recorded pressure-changes (dP) and volume-changes (dV) were substituted into the general formula for the determination of the coefficient of elasticity—$E = dP \Big/ \frac{dV}{V}$. The total volume ($V$) was taken to be, as in previous experiments, the contents of the dural sac, both cranial and spinal. The results of the series of tilts are given in table 1, together with the derivations of the fraction dV/dP on head-down and tail-down tiltings. The coefficient of functional elasticity, when calculated on this basis with use of the general average of the fraction, was found to be 4.70 $\times$ 10^5 dynes per cm.^{2}

As shown in table 1, head-down and tail-down derivations of the fraction dV/dP, when computed from the findings of the 4 mm., 6 mm., and 8 mm. manometers in comparison with the 1 mm. manometer, were almost exactly equal—0.669 and 0.665. The changes recorded for the larger manometers (10 mm., 12 mm., 15 mm.) could not be employed for the

determination of the average value of the fraction, as all of these manometers showed that the volume-dislocation into or from the subarachnoid space had reached an apparent limit: the fraction therefore decreased on both types of the tilting. This phenomenon of maximum volume-dislocation is in accord with the previous findings (3).

The table given represents the second series of tilting experiments performed on the animal, although the anesthesia was continued uninterruptedly. The first series of readings was rendered inaccurate by the animal sneezing and retching on the head-down tiltings though completely anesthe-

TABLE 1

Derivation of dV/dP and of E for Chimpanzee F4

MANOMETER BORE	HEAD-DOWN					TAIL-DOWN				
	Pressure-change C.S.F.	Difference in pressure-change	Volume dislocated	Difference in volume dislocated	$\frac{dV}{dP}$	Pressure-change C.S.F.	Difference in pressure-change	Volume dislocated	Difference in volume dislocated	$\frac{dV}{dP}$
mm.	*cm.*	*cm.*	*cc.*	*cc.*		*cm.*	*cm.*	*cc.*	*cc.*	
1	8.0	—	0.154	—	—	6.4	—	0.123	—	—
4	7.0	1.0	0.805	0.651	0.651	5.6	0.8	0.644	0.521	0.651
6	5.8	2.2	1.665	1.511	0.687	4.6	1.8	1.320	1.197	0.665
8	4.1	3.9	2.763	2.609	0.669	3.3	3.1	2.224	2.101	0.678
				Average	0.669				Average	0.665
10	3.7	4.3	2.637	2.482	0.577	2.9	3.5	2.066	1.943	0.555
12	2.6	5.4	2.964	2.810	0.520	2.0	4.4	2.280	2.157	0.490
15	1.5	6.5	2.841	2.687	0.413	1.2	5.2	2.273	2.150	0.413

General average dV/dP (computed from first four manometers on head-down and tail-down tiltings) = 0.667. Intradural volume = 318.4 cc. (cranial, 313 cc.; spinal, 5.4 cc.). $E = V \Big/ \frac{dV}{dP} = 4.70 \times 10^5$ dynes per cm.^{2}. (In C. G. S. units, dP = height in centimeters $\times$ cceleration of gravity (980) $\times$ density (1.006). Therefore $E = \frac{318.4}{0.667} \times 980 \times 1.006 = 4.70 \times 10^5$ dynes per cm.^{2})

tized; on the tail-down tiltings no physiological disturbances were noticed. The tail-down tiltings in this first series yielded a value of dV/dP of 0.668, which is quite like the values obtained in the second series; the head-down tiltings of the first series, in spite of the difficulties of measurement due to the animal's condition, gave a fraction of 0.619.

The findings assume importance because of the equality in the values of the fraction dV/dP on tail-down and head-down tiltings, showing that in this anthropoid, as in the three other mammals used, the coefficient of functional elasticity is the same in the two vertical tiltings. Furthermore, the magnitude of the coefficient of elasticity (4.70×10^5 dynes per cm.^{2}) is quite similar to the findings in the series of dogs, cats and macaques (1, 2,

4). The dental formula of this chimpanzee was found to be the same as that of the Pithecus sinicus C55, included in the previous report (2); in the bonnet macaque the coefficient of elasticity was recorded as 4.46×10^5 dynes per cm.^{2} The coefficient of cerebrospinal elasticity in this juvenile chimpanzee therefore falls well within the values reported for the group of juvenile macaques.

SUMMARY

The coefficient of functional elasticity of a juvenile chimpanzee's dural sac and its contents was determined by measurement of the pressure-changes and volume-changes of the cerebrospinal fluid on vertical tiltings and by substitution of these measurements into the general physical formula for determination of elasticity $\left(E = dP \Big/ \frac{dV}{V}\right)$. The coefficient of elasticity was found to be 4.70×10^5 dynes per cm.^{2}, a value quite similar to that of macaques of similar age.

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STUDIES ON THE PHYSIOLOGY OF SLEEP

IX. Motility and Body Temperature during Sleep

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Since Szymansky introduced his methods of recording activity in animals and man numerous studies were made of motility during sleep. The means employed for registering motility were not always the same, and there were also differences in the treatment of the results. Some investigators used devices which made electric contacts when the bed (and presumably the sleeper) moved, a signal magnet record showing the frequency of motility, while others connected the bed or the bed spring directly to a mechanical recording system and thus obtained not only the frequency but also the amplitude of individual movements. Perhaps the most extensive investigation of the subject was made by Johnson and his co-workers who, in addition to other methods, used a camera that photographed the sleeper each time he moved (Johnson, Swan and Wiegand, 1930). In tabulating their results these workers expressed the time spent in motility during the night as so many five-minute periods in which the sleeper moved once or more. Thus Johnson (1927) states that their "most typical subject, if he stays in bed eight hours, spends about an hour and twenty minutes of that time in stirring every five minutes or oftener," and that by sleeping 8 hours, instead of $7\frac{3}{4}$ hours, their student-subjects "rested more quietly," "the average increase in the length of the rest period being about one-fourth." In another article Johnson (1928) makes the opposite conclusion for adults of middle age, declaring that "it is not unusual for a person to take nearly as much rest on *most* nights of $6\frac{1}{2}$ hours as in the *average* night of $9\frac{1}{2}$ hours." If Johnson and his associates divided the night into one or two minute, instead of five minute, periods, their subjects would be found to have moved during a smaller percentage of all the periods, and the opposite would be the case, if the unit time intervals were lengthened. We therefore decided to reinvestigate the subject and to determine as closely as possible the *actual* time spent in stirring during sleep. We also wanted to record the temperature of the sleeper in order to detect a possible connection between motility and the temperature level or temperature changes during sleep.

Methods. The motility of the sleeper was studied indirectly through

the movements of the bed spring which was either of the vertical multiple coil type or of the net (hammock) type. In either case a vertical rod transmitted the movements of the spring to a strong rubber membrane

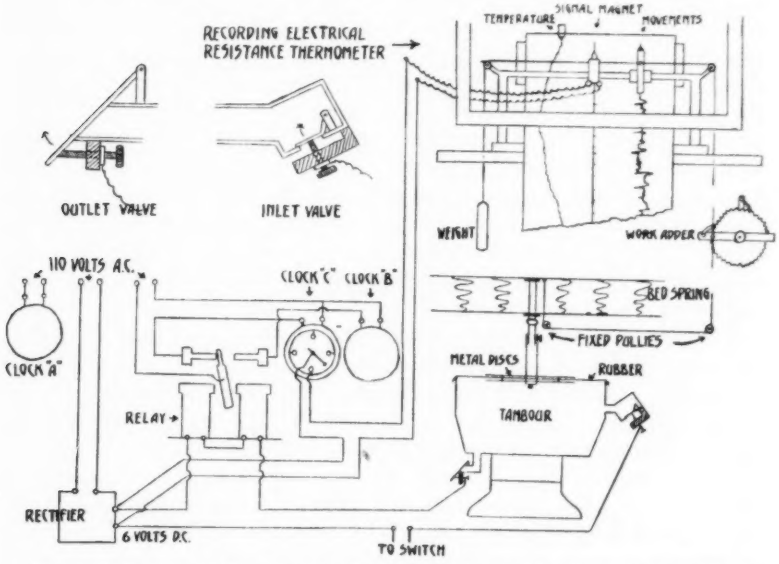

Fig. 1. A diagram of the apparatus for determining and for recording motility and rectal temperature during sleep. The opening of the inlet or outlet valve of the tambour by the movement of the bed spring breaks and keeps open the 6 volt D.C. circuit through the relay, causing the 110 volt A.C. current to start clock *C*, which is not running while the sleeper is immobile, and simultaneously interrupting the flow of current through clock *B*, which has been running all the time and which was set with clock *A*, the latter in no way affected by the movements of the bed spring. The time run up by clock *C* or the time lost by clock *B* in comparison with clock *A* constitutes the total time spent in motility during the night. A string leading from the bed spring over pulleys to a pen recording on uniformly moving paper of the electrical resistance thermometer permits the registration of the extent and frequency of the individual movements, a weight keeping the string taut at all times and insuring a faithful recording of the movements of the bed spring. These movements are transmitted to the work-adder in a unidirectional manner by the same string that leads to the writing pen. The temperature record is made by a similar pen of the electrical thermometer, the connection from the thermometer to the sleeper as well as the construction of the thermometer being omitted from the diagram. Between the two recording pens is a signal magnet activated by the contact between the second-hand of clock *C* and each of the four contact points placed at the 15, 30, 45, and 60 second marks, thus giving a record of every 15 seconds of motility as they are severally run up during the night.

In the upper left hand corner are the details of the inlet and outlet valves of the tambour.

covering a large tambour (30 cm. wide and 10 cm. high), placed directly on the floor and provided with inlet and outlet valves. A downward movement of the spring, compressing the air, would keep the outlet valve open, and during the upward movement of the spring the inlet valve would remain open (fig. 1). When either of these valves was open an electric circuit through a relay was broken, and this break caused a Telechron electric clock, *C*, to start and run until the relay circuit was made again by the cessation of the movement and the closure of the valves. With the clock set at zero (12 o'clock) at the time of going to bed, it was only necessary to read it next morning to find out how much time the sleeper spent in stirring during the night. The inertia of the clock is such that it continues to run for a fraction of a second after the current to it is cut off by the closure of the valves, and the time values read off are higher than they should be. For comparative purposes such a single clock system would do, but to get as close as possible to the true time values we introduced another clock, *B*, which runs continuously, but is stopped while the valves are open. Using clock *B* one sets it according to a third clock, *A*, which is in no way connected with the apparatus and is therefore uninfluenced by the movements of the bed spring, and the time lost by clock *B* compared to clock *A* should be the time spent in motility. With no inertia in the motors of the clocks, the figures obtained by the use of clocks *B* and *C* should be the same, but just as clock *C* furnishes figures that are too high, the figures derived from clock *B* are too low. We were able to calibrate our clocks by introducing a signal magnet into the circuit and recording the duration of the movements on a rapidly moving kymograph, with the time marked off in seconds. It was then possible to obtain factors by which the figures derived from clocks *B* and *C* were to be multiplied to give the time actually spent in movement. The greater the number of short-lasting movements the greater was the difference between the figures furnished by the two clocks, and the employment of two clocks, instead of one, can thus be a source of additional information concerning the type of motility that prevailed during the night. When the clocks are used no registration system of any kind is needed. The apparatus can be placed under the bed in one's own bedroom, and, if so desired, the clock or clocks may be kept in a distant room. The setting and reading of the clocks are all the manipulations required of the observer, and a source of alternating (regulated) current for the Telechron clocks is all that must be provided for the working of the device. Instead of the tambour, a spirometer partially filled with a non-drying liquid can be used, with two pipes leading to the air space of the spirometer terminating in inlet and outlet valves respectively.

To get, in addition to the time spent in motility, the distribution of the movements through the night, a slow-moving kymograph can be set up in a distant room, the face of clock *C* provided with four contact points at the

15, 30, 45, and 60 second marks, and each of these points connected in series with a source of current, a signal magnet, and the second-hand of the clock (fig. 1). Whenever 15 seconds of movement are completed, the second-hand completes the circuit with one of the contact points, and the signal magnet records it on the moving paper. We have used this device in connection with our clock system.

Our experiments have so far been conducted in the laboratory, and to compare the frequency with which our subjects stirred with the figures reported by others we also used a mechanical registration system by means of which a Keith Lucas lever recorded the actual displacements of the bed-spring on kymographic paper. The record showed not only the frequency, but also the amplitude of the displacements of the bed-spring. The sum of all the recorded displacements gives an indication of the extent of motility during the night, and this can be obtained with difficulty and little accuracy by measuring and adding the individual strokes. To get this information with ease and accuracy we made the string that led from the bed-spring to the writing lever operate a Harvard "work-adder," provided with a revolution counter (fig. 1). Setting the counter at a certain figure before going to bed and reading it the next morning, one gets the number of revolutions of the work-adder wheel, to a small fraction of a turn, and knowing the length of one circumference of the wheel it is easy to determine the sum-total of all the displacements. The work-adder device, the simplest and cheapest of all we have used, can be clamped to the frame of a bed without the sleeper's knowledge, requires no energy to operate, and should be very useful for comparative studies where the total motility is wanted, rather than the distribution of movements.

While the sleeper was usually passive in this study, in some cases he was instructed to press a key attached to his bed, if he woke up during the night, thus making a signal magnet record of the fact. In this wise it was hoped to obtain information concerning motility preceding and following awakening.

A continuous record of the rectal temperature of the sleeper was made by means of a Leeds and Northrup electrical resistance thermometer, which permitted us to discern temperature differences of 0.02°F. The rectal piece was 6 cm. long and 4 mm. in diameter and was provided with a flange to prevent it from slipping out. Before insertion it was dipped in glycerin, and the subject not only experienced no discomfort, but was often entirely unconscious of its presence in the rectum. The cord connecting it with wall registration box was sufficiently long to permit the sleeper to turn over without being restrained in his movements. Objectively no difference in the frequency, distribution or extent of motility was noted whether or not the subject's temperature was recorded during his sleep. When the thermometer was used the mechanical registration of movement and the

15-second signal magnet record were made on the same paper on which the temperature was recorded, all three pens writing in line with each other. This paper was moved by a synchronous motor and was ruled for half-hour intervals, making it possible to dispense with a time marker. The arrangement permitted us to relate motility to temperature changes, should such a relation be found to exist.

Eight male subjects were employed in this study. Most of the observations were made on ourselves who served as subjects from 40 to 100 nights. On the others we obtained but few data, but as their records confirmed in every way the information derived from the more extensive observations on ourselves, the combined results may be considered as indicating general rather than individual characteristics of sleep.

RESULTS. The time actually spent in motility was much smaller than expected on the basis of the frequency with which movements occurred

TABLE 1

The average number of movements during the night and the percentage occurring during the second half of the night

SUBJECT	NUMBER OF NIGHTS	NUMBER OF MOVEMENTS			PERCENTAGE FALLING IN SECOND HALF OF NIGHT		
		Major	Minor	Total	Major	Minor	Total
B. O. B.	6	14.2	25.2	39.4	71	63	66
N. B.	6	16.4	18.7	35.1	65	65	65
E. B.	5	20.8	34.1	54.9	66	69	68
N. R. C.	86	27.5	19.5	47.0	58	55	57
A. I. D.	5	11.0	11.5	22.5	62	69	66
N. K.	11	22.6	13.5	36.1	61	59	60
F. J. M.	66	12.8	28.3	41.1	66	63	64
S. P.	5	16.4	23.3	39.7	67	60	63

during the night. In extensive series of observations on three subjects the time taken from rest by movements of all kinds, for each hour of staying in bed, was found to be, on the average, 43.0 seconds (K., 38 nights), 34.1 seconds (C., 66 nights) and 19.7 seconds (M., 59 nights). From two to four minutes was all that was not spent in complete immobility during the usual night's sleep. This time was not equally distributed through the night. By having each 15 seconds of motility recorded by means of a signal magnet it was shown that the greater part of this time was spent in stirring during the second half of the night. Subject M (15 nights) spent 67 per cent of the total time in moving during the second half of the night.

The sum of all the displacements of the bed spring in one direction as determined by the work-adder and counter arrangement was found to vary in the same sense as the time spent in motility. For the above-mentioned three subjects the average hourly displacements were 15.4, 14.0 and 7.4

cm. corresponding to hourly motility of 43.0, 34.1 and 19.7 seconds. Attempts to develop a formula whereby work-adder readings could be translated into time were not successful, but when clocks are not available the work-adder device can be used to get an approximation of the time spent in movement, each centimeter of displacement being equivalent to about 2½ seconds of motility.

The work-adder does not give any information concerning the distribution of the movements; that was obtained from the mechanical record on the kymograph or thermometer paper. As pointed out by other workers, it is impossible to tell from the record what the movements were, but we divided all the excursions of the writing points into two groups, signifying major and minor movements, the first, as determined by trial, due to turning over or to a change in the position of the entire body, the second, to the movement of a part of the body. While not exact, this division gives an indication of the distribution of motility as regards the magnitude of movements. In six out of eight subjects the minor movements outnumbered the major ones. The average number of major movements per night in different subjects varied from 11.0 to 27.5, and the minor movements, from 11.5 to 34.1. The total number of recorded movements was, on the average, 22.5 for the most quiet sleeper, and 54.9 for the least quiet. In all eight subjects, for both major and minor movements, the greater number of movements occurred during the second half of the night (table 1).

There was a complementary distribution of quiescence during the night's sleep. We examined our records for the distribution of periods of complete immobility lasting 30 minutes or more and noted that in the case of subject C. these occurred from 1 to 3 times per night, with an average of 2.1 for 48 nights; for K. the variation was from 1 to 6 such rest periods per night, with an average of 3.1 for 19 nights, and for M., from 3 to 8, with an average of 5.6 for 30 nights. The greater number of these long periods of immobility fell in the first half of the night, the percentages of the total being 56.5 for C., 55.2 for K., and 66.5 for M. There was thus an inverse relationship between the number of movements and the number of periods of rest of 30 minutes or more in the two halves of the night.

The figures for the frequency with which one woke up during the night and was sufficiently awake to remember to press the signal magnet showed that this was done more frequently during the second half of the night. Some subjects were at no time sufficiently aroused during the night to perform this simple task, others did it but had no recollection of the fact next morning. The systematic investigation of this subject was limited to one person, M., who pressed the key 101 times in the course of 20 nights, on the average 5 times per night. Of these instances of awakening 0.8, on the average, took place in the first half of the night, and 4.2 in the second. The awakenings were always accompanied by a movement, which during

the first half of the night was always of the major type, and during the second half of the night, mostly, but not always, of that type. The tendency to awaken spontaneously during the night was found to have a direct relationship to one's ability to be aroused by extraneous stimuli, but not to quiescence or motility during sleep. Thus subject M. awakened more frequently and could be aroused more easily than subject C. whose frequency of stirring was of a higher order.

The temperature changes are of interest mainly because they were recorded synchronously with motility during sleep. The temperature curves themselves did not differ from those published by Benedict and Snell (1902) and by others (Piéron, 1913). The most characteristic drop in temperature occurred shortly after going to bed and was undoubtedly due to the assumption of the horizontal position (Kleitman and Doktorsky, 1933). In some cases the subject was instructed to get up during the night and stand up for an hour. A characteristic rise in the temperature curve always occurred during standing, followed by an equivalent drop after the horizontal position was resumed. Examining individual records it was found most convenient to compare the temperatures at the end of successive half hour periods after going to bed, rather than according to the time of the night. There were considerable differences in rectal temperature from night to night, both at the time of going to bed and during sleep, the fluctuations varying from one-half to more than one degree F. for same half-hour on different nights.

There seem to be four personal characteristics in the temperature variations, as was found by a study of 74 nightly records of two subjects (M.,

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Fig. 2. Temperature and motility of two subjects during the summer-autumn and winter-spring seasons. The upper four curves are based upon data obtained on subject M., 20 nights in summer-autumn and 13 nights in winter-spring, and the lower four, on subject C., 14 nights in summer-autumn and 27 nights in winter-spring. The temperature curves are drawn through the average values at the end of successive half-hour periods after going to bed. The dots above and below each temperature curve represent the range of temperature variability, i.e., the maximum and minimum temperatures for the particular half hours during the several nights, and not the highest and lowest individual nocturnal temperature records. The temperature level, the mean of all the points in the average temperature curve, is 98.52 degrees in summer-autumn and 98.28 degrees in winter-spring for M., and 98.45 and 98.06 are the corresponding values for C. It will be noticed that the only temperature points common to both seasons, for each subject, are the maximal temperatures at the time of going to bed and shortly thereafter, the minima for the corresponding half hours varying widely in the two seasons.

Under each temperature curve is plotted the curve of motility for the particular season, based on the average number of movements during successive hours after going to bed. The average total number of movements per night, for M., was 40.1 in summer-autumn and 39.1 in winter-spring, and for C. the figures were 53.7 and 44.5 respectively.

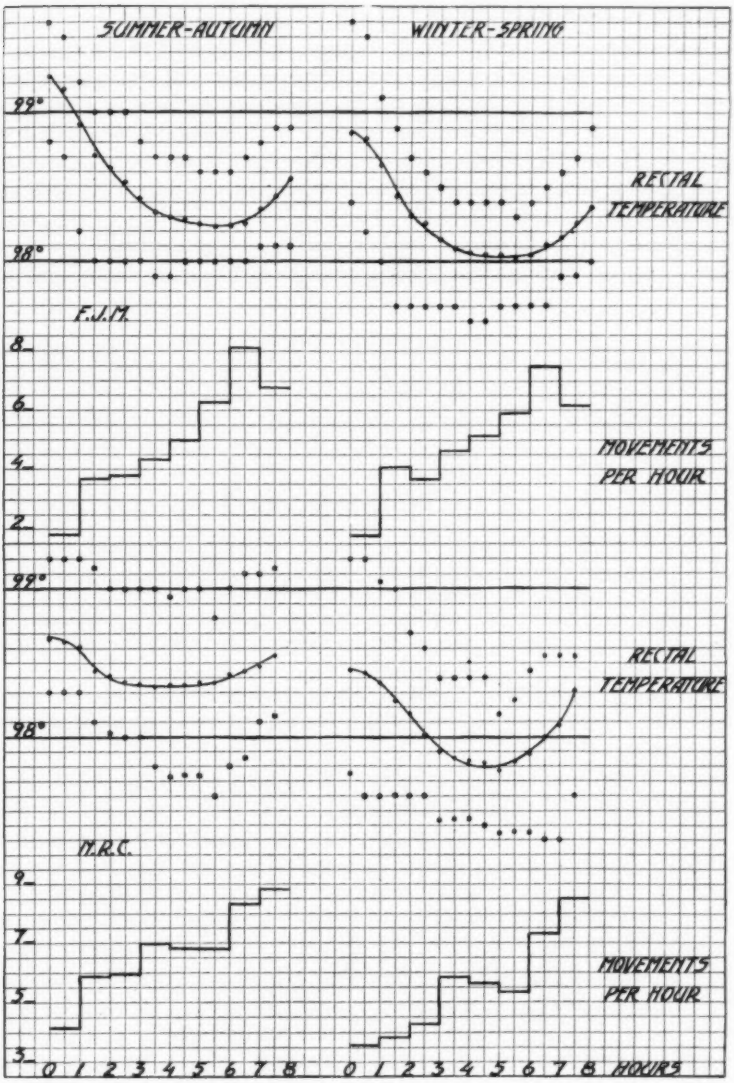
SUMMER-AUTUMN
WINTER-SPRING
99°
98°
RECTAL TEMPERATURE
F.J.M.
8
6
4
2
MOVEMENTS PER HOUR
99°
98°
RECTAL TEMPERATURE
M.R.C.
9
7
5
3
MOVEMENTS PER HOUR
0 1 2 3 4 5 6 7 8
0 1 2 3 4 5 6 7 8 HOURS

Fig. 2

33 nights, C., 41 nights), and they are shown in figure 2. These are: *1*, the nocturnal temperature level, obtained from the average temperatures for successive 30 minute periods, 98.42 degrees for M. and 98.19 for C.; *2*, the average temperature range for corresponding times on different nights, 0.85 degree for M. and 1.10 for C.; *3*, the average drop from the time of going to bed to the time the lowest temperature of the night is reached, 0.95 degree for M. and 0.50 for C.; and *4*, the difference between the temperature at the time of going to bed and that prevailing at the time of getting up, 0.60 degree for M. and 0.10 for C. The parallelism existing between *3* and *4* may be related to the diurnal temperature curve and the time its highest point is reached during the waking hours, the latter being about one-half

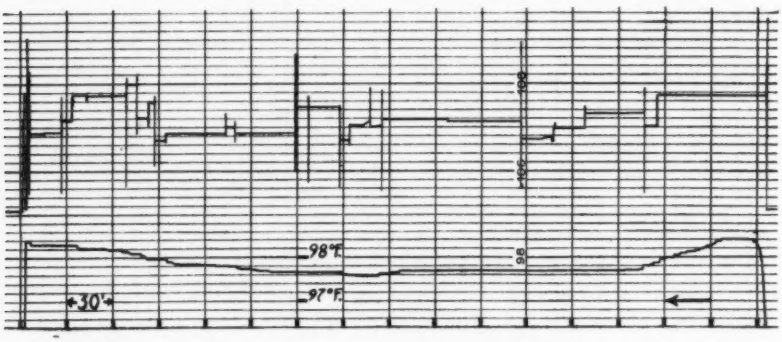

Fig. 3. Reduced reproduction of an actual record of motility (above) and rectal temperature (below) made by subject M. The signal magnet record is omitted.

a degree higher and reached at a much later hour of the day in M. compared to C. (see Kleitman, 1933, fig. 2).

There was no definite connection between motility and temperature changes. In some cases a change in position was followed by a marked change in temperature, but that may have been due to the shifting of the thermometer piece in the rectum. In figure 3 are shown simultaneous and synchronous records of motility and temperature (with the signal magnet record left out), and it will be noticed that considerable motility may take place without a change in temperature, and vice versa. Generally speaking, there are fewer movements during the downward course of the temperature curve than during its upward swing. There seems to be no relation between the general temperature level and motility in different subjects. Thus subject M. has a higher temperature level and a lower motility than subject C.

Each of the two subjects showed a seasonal variation in both motility and temperature, in the latter more definitely than in the former. The observations scattered over more than a year were grouped under two headings: summer-autumn (May 17–Nov. 16) and winter-spring (Nov. 17–May 16), but most of the data were accumulated in the autumn and in the winter. From figure 2 it is evident that temperature level and motility are higher in the autumn than in the winter. As would be expected similar differences appeared in the figures obtained by means of the clocks and the work-adder device.

Discussion. During a night's sleep our subjects executed, on the average, from 11 to 28 major movements, probably involving a change in the position of the sleeper, and the average total number of movements varied from 22 to 55. These findings are in agreement with the statement of Johnson and associates (1930) that their most typical subject assumes from 20 to 49 different positions in the course of a typical night of eight hours. However, these investigators did not observe any constant distribution of the movements through the night, except as characterizing the sleep of an individual subject. Thus Johnson, Swan and Wiegand (1926), analyzing the results obtained on eleven subjects, concluded that there were personal differences in motility, "some subjects tending to rest more during the first half of the night; others during the last half; others during the middle." Our subjects uniformly moved more frequently during the second half of the night, the ratios for the two halves varying from 1:2 to 2:3. When plotted for successive hours during the night (fig. 2) the curve of motility is irregularly ascending through the entire period of sleep. Similar curves were obtained by Dr. Glenville Giddings in his as yet unpublished study of the motility of school children during sleep.

Our clock readings show that the total time "wasted" in motility is a matter of but a very few minutes per night. That, of course, does not mean that the subject is awake during the time he executes the movements, nor that he is asleep during the rest of the time. Concerning the former, we have the signal magnet records made when the subjects awoke during the night sufficiently to press the key. Subject M., who moved, on the average, over forty times per night, pressed the signal magnet key, on the average, only five times. There are thus few interruptions of sleep during the movements, and the time that should be deducted from sleep need not be as great as the time spent in stirring. Just how long, on occasion, the sleeper may be awake before or after a movement we have no means of knowing, but we know that he was lying quietly, if awake. In other words, from our clock readings we know that except for a very few minutes the subject rested completely, in the sense of not moving, during the entire night, whether he was asleep or awake. Aware from personal experience that when one is awake during the night one generally stirs a good deal,

we feel certain that our subjects were asleep practically all the time they were at rest. We do not consider as justifiable to count a five minute period "wasted," if a movement lasting a few seconds occurred during that period. Nor can we see how one can increase his rest 25 per cent by staying in bed 8, instead of $7\frac{3}{4}$ hours.

Our body temperature findings are characterized by considerable differences in temperature at the same hour after retiring in a given subject on different nights. Yet the average nocturnal temperature curves show definite personal characters as regards shape and general level. In normal sleep there seems to be no definite relationship between temperature level or variations and the number or frequency of the movements executed by the sleeper. There is a suggestion that a higher temperature may mean a greater inclination to spontaneous awakening and ability of being aroused by outside stimulation. There is also an indication of a parallel seasonal variation in both temperature and motility, and we shall shortly publish data that show that in a given subject changes in the temperature level during sleep accompany (cause? are caused by?) similar changes in motility.

SUMMARY

1. New methods of studying motility during sleep are described.

2. The time actually spent in movement during sleep is very small, about half-a-minute per hour.

3. There is a gradual increase in frequency of movement during sleep, so that one moves considerably more during the later than during the earlier part of the night.

4. A continuous recording of the rectal temperature of the sleeper synchronously with motility shows no definite connection between the temperature changes and motility.

5. In individual sleepers there may be a seasonal variation in the temperature level and a parallel change in motility.

We are greatly indebted to Messrs. B. O. Barnes, N. Brewer, E. Borkon, A. I. Doktorsky, and S. Platt for their valuable assistance in obtaining the data reported.

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EFFECT OF DIET ON RESPONSE TO PARATHYROID EXTRACT AND VITAMIN D

I. The Relation of Calcium and Phosphorus of the Diet to Response to Parathyroid Extract in Rats

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A definitely decreased response to injection of parathyroid extract as expressed by occurrence of little or no rise in serum calcium and absence of other accompanying symptoms was observed in this laboratory recently (Morgan and Garrison, 1) in young dogs which had been reared on vitamin D-free diets of moderately high Ca:P ratios. A few observations upon similar animals fed diets of considerably lower Ca:P ratio indicated that on such diets the differences in degree of hypercalcemia exhibited by vitamin D-free and vitamin D-rich dogs were smaller than those seen in the earlier experiments. It seemed worth while to investigate the possible relation of the calcium and phosphorus content of the diet to the effect of parathyroid treatment both in the presence and in the absence of the antirachitic vitamin. Furthermore the possibility of extending these observations to another species, the rat, appeared desirable in view of the conflicting opinions as to the relative immunity of this species which have appeared in the rapidly growing literature concerning the parathyroids.

Tweedy and Chandler (2) found the normal rat quite resistant to parathyroid treatment as did Bauer, Aub and Albright (3). The latter investigators noted similar lack of response in cats but found normal rises in the serum calcium of rabbits when moderate doses of the hormone were administered. Rose (4) has recently shown, however, that normal and vitamin B-deficient rats when given 25 units of the extract per 100 grams of body weight daily are likely to succumb after three doses. This is a very large dosage however as compared with clinical values or those usually used in experiments on dogs.

The effect of parathyroid extract treatment upon bones and other body tissues of rats has been studied by Lambie, Kermack and Harvey (5) who found the trabeculae much thinned in rats which had received 10 units daily for 21 days but an increase in the calcium content of the ash of the bones, although the total ash of these bones was reduced. Bauer, Aub and Albright (3) found on the other hand an increase in the bony trabeculae

in their parathyroid treated rats although the size of the total bony structure was decreased below that of untreated controls. Day (6) analyzed the whole bodies of young rats which had received 15 units of the extract daily from 21 or 28 to 50 days of age and noted no change in either calcium or phosphorus content from that shown by the untreated controls. In most of these reports there is little detail as to the diet given the ani-

TABLE 1

Composition of the diets

DIET NUMBER	TYPE	CONTENT	Ca	P	Ca : P RATIO
			per cent	*per cent*	
A	Steenbock rachitogenic (high Ca-low P)	Ground yellow corn..... 76 Wheat gluten..... 20 $CaCO_3$..... 3 NaCl..... 1	1.27	0.27	4.7
B	Normal	Wheat gluten..... 10 Egg albumen*..... 10 Agar..... 2 Crisco..... 15 Dextrin..... 59 Salts no. 5**..... 4 Yeast, 0.5 gram daily Alcoholic extract of spinach equivalent to 0.5 gram spinach daily	0.49	0.36	1.3
C	Low Ca	The same as diet B except that salts no. 4‡ was used instead of no. 5	0.10	0.42	0.2

* Commercial dried egg albumen of Chinese origin extracted three times with boiling absolute alcohol.

** Salts no. 5 was made up as follows:

KH_2PO_4	18.5
$CaH_4(PO_4)_2 \cdot H_2O$	51.2
$MgSO_4$	4.5
NaCl	20.4
Ca lactate	89.7
Ferric citrate $+\frac{1}{2}H_2O$	2.0

‡ See Morgan and Garrison (1).

mals and in no case is a variation in the mineral composition of the diet included in the plan of experiment.

The diets used in the study here reported were three in number, of high calcium-low phosphorus, normal and low calcium-high phosphorus content. The composition of these diets is shown in table 1. In each series of experiments each of these diets was fed simultaneously to two groups

of young rats from our own colony beginning at the ages of 21 to 35 days and continuing for varying periods. One group was given daily additions of potent cod liver oil or viosterol, the other was given no source of antirachitic vitamin and was kept in the dark. During the last three days of the period graduated doses of parathyroid extract[1] were injected subcutaneously into one-half of each group of rats. All the animals were then killed, the blood of each group pooled and analyzed for serum calcium and inorganic phosphate by the usual methods (1). The tibia of the left leg was dissected out, split, treated with silver nitrate and observed for evidences of calcium deposition in the epiphysis, according to the line test technique of McCollum, Simmonds, Shipley and Park (7). The right femur was separated from attached tissues, dried, extracted with alcohol and ether and ashed in a muffle furnace. There were thus assembled data as to ash content of the femora of the individual animals as well as the total calcium and inorganic phosphate phosphorus of the pooled sera of the groups.

The diets. The diets were chosen in such a way as to control their ash content as closely as possible. Thus in diets B and C wheat gluten and egg albumen were included instead of casein because of their low calcium and phosphorus contents and spinach extract was used as source of vitamin A so as to introduce as little ash as possible. The egg albumen was extracted three times with boiling absolute alcohol as is usually done in the preparation of vitamin A-free casein in order to remove any traces of vitamin D.

The salt mixture used in the normal diet (B) is based on the composition of dog milk salts and was originally devised for use with growing puppies. Its calcium and phosphorus content are not much different from that of the salt mixtures of Osborne and Mendel and McCollum's 185 which have been so extensively utilized for growing rats. The low calcium salt mixture of diet C is an adaptation of the Osborne and Mendel mixture to calcium-free condition. The calcium and phosphorus levels in diet B are close to the levels recommended by McCollum, Simmonds, Kinney, Shipley and Park (8) as optimal for rat growth. The phosphorus level of diet C is similar but the calcium is lowered considerably. Nearly normal growth was secured on diet B both with and without added source of vitamin D and in some cases also upon diet C with vitamin D, but on diet C without vitamin D the animals were stunted, often deformed and short-lived. Because of the severity of the diet the feeding period on diet C was decreased to

[1] Most of the parathyroid extract as well as the cod liver oil and viosterol used in this study was kindly furnished by E. R. Squibb and Sons. Each of these materials was tested for potency, by the line test in rats in the latter two cases and by the rise in serum calcium of normal dogs in the first case.

50 or 51 days instead of the 70 to 80 days originally planned and carried out with diet B.

As source of vitamin D cod liver oil or viosterol was used, the latter in two groups, 5 and 6 on diet A and one on diet B, the former in all other groups. One drop (averaging 25 mgm.) of a potent cod liver oil (Squibb's) or one-tenth drop (2.5 mgm.) of viosterol (Squibb's 100 D) was given each rat separately on each day.

Parathyroid extract of known potency was administered subcutaneously to certain groups as follows, 20 units each day for the last 3 days before sacrifice of group 1 on diet A, 10, 20 and 30 units respectively on the last 3 days of life of groups 2, 3 and 4, and 10, 20 and 10 units for groups 5 and 6 on diet A, 20, 30, and 40 units to all groups on diet B, 10, 20 and 30 units to group 1 on diet C and 20, 30 and 40 units to the other groups on diet C. The higher doses were given the rats on diets B and C because it was early apparent that these animals were more resistant to the influence of the hormone than were similar animals fed diet A with vitamin D.

The blood serum values. It is plain that on diet A, of high calcium to phosphorus ratio, the groups which received vitamin D had more marked rises in serum calcium than did those without the vitamin when similar doses of parathyroid extract were administered. The size of the average increases (table 2), 1.4 mgm. per 100 cc. in the one group and 0.2 in the other, leaves little doubt that vitamin D exerts a dominating influence on this response. Since the probable error of the means varies from 0.2 to 0.5, the former increase may be considered significant but the latter insignificant.

The inorganic phosphate of the serum is seen to be raised about 1 mgm. per 100 cc. by the administration of the parathyroid extract in groups on diet A both with and without vitamin D. This is sufficient in the latter group to raise the Ca $\times$ P product above the level of 30, which has been suggested as the critical figure above which calcification takes place. Kramer, Shear and Siegel (9) have recently shown that when the Ca $\times$ P product is raised above 30 by any means new calcification occurs in rats with low phosphorus rickets. Healing, preceded by this increase in the product is produced in three days by administration of cod liver oil or viosterol, in 24 hours by fasting and in 12 hours by addition of phosphate to the diet. In the study here reported the rats without vitamin D to which parathyroid extract was given for three days preceding sacrifice showed distinct healing as indicated by the line test, illustrated in figure 1, as well as Ca $\times$ P product of 36 as compared with active rickets and Ca $\times$ P product of 25 in the corresponding untreated group.

On diet B, of normal Ca to P ratio, normal serum calcium and inorganic phosphate figures were found in all groups. An increase of 1.3 mgm. per 100 cc. in calcium was produced by parathyroid extract when vitamin D was present and of 1.8 when no vitamin D was given. A similar difference,

1.4 mgm. per 100 cc., is seen between the groups with and without vitamin D but without parathyroid treatment. No significant change in inorganic phosphate appears ascribable to the hormone effect when vitamin D is present but in the absence of vitamin D a lowering is produced.

On diet C, of low Ca to P ratio, average increases of 0.4 mgm. per 100 cc. when vitamin D was given and of 1.6 without the vitamin occurred in the

TABLE 2

Effect of parathyroid extract and vitamin D upon serum calcium and phosphorus and femur ash of rats fed diets of varying Ca:P ratio

DIET	NUMBER OF GROUPS	TOTAL NUMBER OF RATS	LENGTH OF PERIOD	AVERAGE WEIGHTS		SERUM CALCIUM	SERUM INORGANIC PHOSPHORUS	FEMUR ASH
				Initial	Final			
			days	*gms.*	*gms.*	*mgm. per 100 cc.*	*mgm. per 100 cc.*	*per cent*
A. High Ca-low P, with cod liver oil and 60 units parathyroid extract.	6	27	31	51	78	14.2	4.5	45 ±1.0
Same, but no parathyroid extract.	6	28	31	47	81	12.8	3.7	44 ±0.9
Same, but without cod liver oil, with 60 units parathyroid extract	6	30	31	49	84	11.3	3.2	39 ±0.9
Same, without cod liver oil or parathyroid extract	6	30	31	52	85	11.1	2.3	38 ±1.5
B. Normal Ca and P, with cod liver oil and 90 units parathyroid extract.	3	9	81	82	167	12.8	5.7	53 ±0.4
Same, with cod liver oil, no parathyroid extract	3	11	79	66	149	11.5	5.3	51 ±0.7
Same, without cod liver oil, with 90 units parathyroid extract	3	14	74	71	144	11.9	5.0	50 ±0.7
Same, without cod liver oil or parathyroid extract	3	13	80	76	134	10.1	6.5	50 ±0.9
C. Low Ca-normal P, with cod liver oil and 90 units parathyroid extract	4	14	51	59	147	12.4	5.9	41 ±0.8
Same, with cod liver oil, no parathyroid extract	4	14	51	70	151	12.0	7.3	44 ±0.9
Same, without cod liver oil, with 90 units parathyroid extract	4	19	51	70	112	8.7	6.3	44 ±0.7
Same, without cod liver oil or parathyroid extract	3	18	50	64	117	7.1	7.5	42 ±0.8

serum calcium upon parathyroid treatment. The difference in serum calcium due to vitamin D alone, however, 4.9 mgm. per 100 cc., was considerably larger on this diet than on either of the other two diets. The inorganic phosphate appeared to be significantly lowered, 1.4 and 1.2 mgm. per 100 cc., by the parathyroid action both with and without vitamin D.

The hypercalcemia of parathyroid origin appears thus to be intensified

by vitamin D on diets of high Ca to P ratio, to be unaffected by vitamin D on diets of normal Ca to P ratio, and to be lessened by vitamin D on diets of low Ca to P ratio. The serum inorganic phosphate is affected by parathyroid treatment in quite the opposite directions, that is, it is raised on diets of high Ca to P ratio, unchanged on normal ratio, unless it may be considered lowered when the vitamin is absent, and lowered on low Ca to P ratio. The significance of these differences is difficult to assess.

The femur ash. In table 2 are shown the amounts of ash found in the fat-free femora of some of the rats used in this study. The amount of ash expressed as per cent of the extracted femora is somewhat higher for the D-free groups on diet A than has been reported by Adams and McCollum (10), 38 as compared with 23 to 34 per cent, but similar to that reported by Bethke, Steenbock and Nelson (11), 39.78 for a similar low phosphorus series. Shohl, Bennett and Weed (12) found 31.6 per cent, Brown and Shohl (13) 39.6 per cent. In the last study with the addition of optimal amounts of irradiated ergosterol, the ash content was seen to rise to 46 or 47 per cent. In our series with cod liver oil or viosterol the average is 44 ± 0.9 per cent. It should be noted that the amount of these antirachitic agents fed was small, 25 and 2.5 mgm. (100 D) per day per rat respectively. In the groups both with and without vitamin D on this diet parathyroid extract administration in the amounts used can be said to have produced no change in ash content of the bones.

The per cent of ash in the femora of the rats fed the normal diet B was 53 ± 0.7 with vitamin D and 50 ± 0.9 without the vitamin. The difference appears to be significant, although the figures are somewhat lower than those usually reported for normal bones of rats at these ages. Bethke, Steenbock, and Nelson (11) found 59.54 for rats 66 days old on stock ration. Brown and Shohl (13) report a maximum of 55.9 on Sherman diet with 0.10 mgm. irradiated ergosterol daily and 57.4 on the same diet without vitamin D. Hume and Smith (14) using a diet quite like ours but for 35 to 80 days longer found 60.1 per cent ash in the leg bones of five rats to which no vitamin D had been given and 61.1 to 61.7 in similar groups of those which had received small amounts of unirradiated and irradiated ergosterol. Again the parathyroid treatment appeared to exert no effect in our series upon the bone ash in either case.

On diet C, of low calcium to phosphorus ratio, a low level of ash content was found in all four groups, 41 to 44 per cent, with apparently no significant differences produced either by vitamin D or by the parathyroid extract. The ash content found by Bethke, Steenbock and Nelson (11) in animals reared on a similar diet was 38 to 41 per cent of the fat-free femora, both with and without cod liver oil additions.

Apparently gross analysis of such bones cannot reveal any small differences produced by parathyroid treatment over short intervals but may

show the effect of long-continued antirachitic measures. Separate analyses of the epiphyseal ends of the tibiae and femora, of the shafts, and of the trabecular mass may yield more satisfactory comparisons and this attempt is now being made in this laboratory.

The trabeculae. Upon treatment with silver nitrate of the freshly cut surfaces of the ends of the tibiae of each of the rats used in this study dark deposits of silver were produced on those portions of the bone which were calcified. This is the usual technique of the "line test" for observation of healing in low phosphorus rickets of rats. It appears to serve also as a means of emphasizing the amount and character of the trabeculae and thus possibly, according to Bauer, Aub, and Albright (3), of the reserve of calcium and phosphorus available in the animal's body. If the parathyroid extract mobilized such reserve calcium some differences should be observable under the binocular microscope in the trabeculae of treated and untreated rats.

On the rachitogenic diet A typical bones showing low phosphorus rickets were found when no vitamin D and no parathyroid extract was administered. Apparently normal bones were produced on the same diet with cod liver oil or viosterol. But in rats which had received the parathyroid extract with vitamin D the number of darkened trabeculae seemed much decreased. Typical examples of this difference are shown in figure 2, a photograph of tibiae of litter mates taken under the binocular microscope. In figure 1 are shown similar tibiae of rats which had not received vitamin D, but one of which, that on the left, had been given 60 units of parathyroid extract in the last three days of its life. A considerable amount of healing had occurred in the latter case. Such healing was invariably found in parathyroid-treated rats on the low phosphorus diet.

On normal diet B well calcified bones were seen both with and without vitamin D. A curious difference in the amount of trabeculae was plainly present, however, the vitamin D-free animals showing the heavy deposits illustrated in figure 4, the vitamin D-fed animals the relatively small amount shown in figure 3. Those rats which were given the parathyroid treatment, the left of each pair, in both cases appeared to be depleted of much of this deposit.

On the low calcium diet C only approximately normal bones were found when vitamin D was given as shown in figure 5, but again some depletion of the already scanty trabeculae appears to occur in the parathyroid treated animals. The same curious deposit of trabeculae (fig. 6) characterizes the vitamin D-free bones produced on this diet as on the normal vitamin D-free diet, but here the deposit is formless instead of regular. Again the effect of the parathyroid extract, seen in the bone on the right, is a relative depletion of the trabeculae with a more or less definite increase in regularity of the remaining deposits.

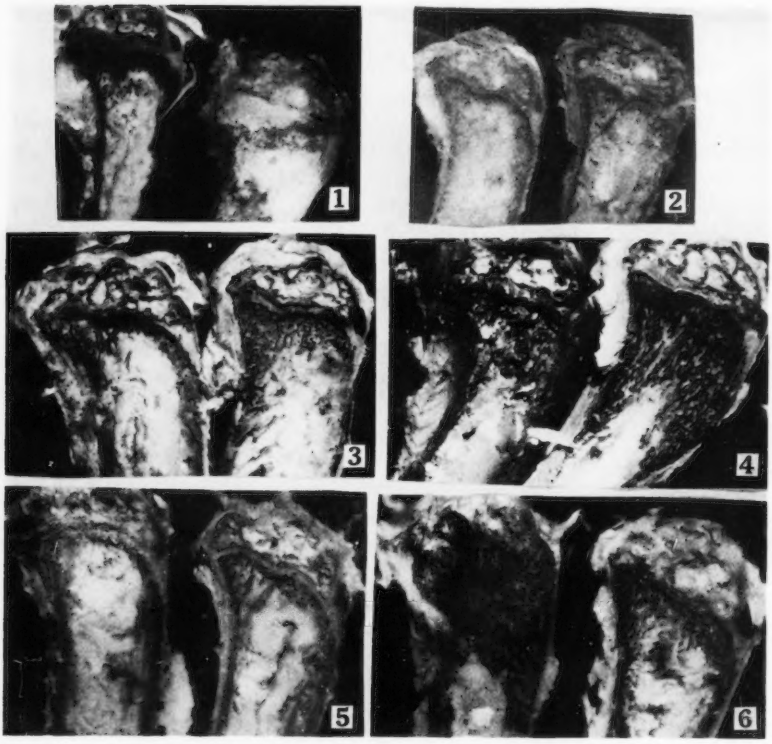

Fig. 1. High Ca-low P diet, without vitamin D.
Left. Sixty units parathyroid extract administered. Note definite healing.
Right. No parathyroid extract.
Fig. 2. High Ca-low P, diet with vitamin D.
Left. Sixty units parathyroid extract administered.
Right. No parathyroid extract.
Fig. 3. Normal diet, with vitamin D.
Left. Ninety units parathyroid extract administered.
Right. No parathyroid extract.
Fig. 4. Normal diet, without vitamin D.
Left. Ninety units parathyroid extract administered. Note depleted trabeculae.
Right. No parathryoid extract. Note excessive but regular trabecular deposit.
Fig. 5. Low Ca diet, with vitamin D.
Left. Ninety units parathyroid extract administered.
Right. No parathyroid extract.
Fig. 6. Low Ca diet, without vitamin D.
Left. No parathyroid extract. Note excessive and irregular trabecular deposit.
Right. Ninety units parathyroid extract administered. Note depleted and reguized trabeculae.

McCollum, Simmonds, Kinney, Shipley, and Park (8) describe in detail the bones of rats fed low calcium diets similar to diet C. They state that "the trabeculae themselves are fairly numerous," "the hyperplasia of the connective tissue about the trabeculae was so great as almost completely to fill the spaces between them and in large part to exclude the marrow." This is the condition observable in figure 6, although the characteristic darkening of the trabecular area which persists after brief treatment with hyposulfite apparently indicates deposition of calcium salts intimately throughout the connective tissue. McCollum, Simmonds, Shipley, and Park (15) describe the microscopic appearance of bones of rats reared on low calcium diets as containing "small, convoluted and very numerous trabeculae, as in bones which show osteosclerosis. These trabeculae are incompletely calcified and the calcium deposits are granular as though they were being rapidly disintegrated and rebuilt."

No histological examination of the bones of the rats was attempted in the study here reported but the macroscopic appearance of the cut surfaces studied was seen to correspond closely with the description cited above.

Bauer, Aub, and Albright (3) report that four young rats kept on high calcium diet and given 885 units of parathormone in 110 days showed denser and shorter bones than their controls and many trabeculae. This is comprehensible if the diet used was vitamin D-free and is paralleled by our observations on diet A as illustrated in figure 1. Since the serum calcium was not raised it seems likely these rats were vitamin D-free. Our finding that the number of trabeculae is reduced under parathyroid treatment on all diets except high calcium without vitamin D supports the conclusions of these investigators.

These results are in line also with those of Bodansky and Jaffe (16) who found that young dogs on low-calcium diets and receiving frequent parathyroid extract injections eventually developed a marked hypocalcemia accompanied usually by hyperphosphatemia. Such animals did not show a rise in serum calcium upon receiving even relatively large doses of the hormone but on autopsy were found to exhibit the typical lesions of ostitis fibrosa cystica. Upon administration of calcium, however, a rise in serum calcium was noted in some of these cases. If the excess calcium and phosphorus usually noted in the serum and said to be excreted under the influence of the hormone is of essentially bone cortex origin it is difficult to understand why the calcium intake should have so notable an effect. The data presented here and by Bodanksy and Jaffe appear rather to point to the calcium and phosphorus of the diet and of an easily mobilized reserve, probably in the trabeculae, as the source of this excess at least when only moderate doses of the parathyroid extract are given.

SUMMARY. 1. Young rats fed diets of varying calcium to phosphorus ratio and content, with and without a source of vitamin D, were given 60

to 90 units of parathyroid extract in gradually increased doses during the last three days of life. Determinations of the "line test," femur ash, serum calcium and inorganic phosphate were made in all cases. Control groups without parathyroid treatment were prepared similarly.

2. On the Steenbock rachitogenic diet of high calcium to phosphorus ratio with vitamin D, the average rise in serum calcium due to parathyroid treatment was 1.4 mgm. per 100 cc., but without vitamin D there was no significant rise. Serum inorganic phosphate rose about 1 mgm. in each case. The line test indicated definite healing in the parathyroid treated rats without vitamin D.

3. On a diet of normal calcium to phosphorus ratio and content, parathyroid dosage produced a slightly greater rise in serum calcium in the animals without vitamin D than in those receiving it. The serum phosphate was lowered in the former group. An unusually large deposit of trabeculae in the tibia was seen in the group without vitamin D and in all those which had received the parathyroid extract depletion of these trabeculae was observable.

4. On a diet of low calcium to phosphorus ratio, parathyroid dosage produced a larger rise in the serum calcium of the groups without vitamin D than in those receiving it, 1.6 as compared with 0.4 mgm. per 100 cc. But the original level of serum calcium was normal in the latter and so low in the former that the parathyroid treatment did not raise it to normal levels. In both cases the serum phosphate was definitely lowered. A large but irregular deposit of trabeculae was seen in the bones of the D-free and but few trabeculae in those of the vitamin D-rich group.

5. In none of the groups did the parathyroid treatment produce a significant effect upon the ash content of the femora, although significantly larger ash per cents were seen in the bones of the rats receiving vitamin D than in those without it in all cases except in that of the low-calcium diet.

CONCLUSION

Parathyroid extract in moderate doses produces a rise in serum calcium only when a good supply of calcium is available from the diet or from an accumulated reserve, probably in the bone trabeculae.

Quantitative comparisons of the effect of parathyroid extract cannot be accurately made without careful control of the calcium and phosphorus content and ratio of the diet as well as of the vitamin D intake. The amount of calcium reserve accumulated and the current demands for calcium and phosphorus in the experimental organism also probably seriously affect the outcome.

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THE EFFECT OF DIET ON RESPONSE TO PARATHYROID EXTRACT AND VITAMIN D

II. The Effect of High Calcium-Low Phosphorus Diets in Dogs

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There was found in this laboratory some time ago a definite decrease in normal response to parathyroid extract injections in young dogs deprived of vitamin D. These experiments were carried out using chiefly the high calcium-low phosphorus type of diet of the nearly normal Ca:P ratios of 1.18 to 1.77 (1). Only two dogs on the low Ca:P ratios of 0.39 to 0.50 were used and the rise in serum calcium in these two cases was found to be definitely less than in similar animals fed diets of higher Ca:P ratio. This discrepancy appeared to warrant an investigation of the rôle of the calcium and phosphorus content of the diet in the effect of parathyroid treatment with and without the addition of some source of vitamin D.

Albright, Bauer, Ropes, and Aub (2) in their extensive study of the effect of the parathyroid hormone on eight clinical cases draw the conclusion that the primary effect is upon phosphorus and that the meat diet may be deleterious in parathyroid tetany because of its high phosphorus content. They used in all cases but one an extremely low calcium diet and obtained as will be noted hereafter the typical low calcium response. In the one case in which calcium lactate was added to the diet a high calcium response in the serum resulted.

Bodansky, Blair and Jaffe (3) found exaggerated response to parathyroid injections in young guinea pigs fasted six hours or longer. They ascribe this to the removal of the normally basic diet of these animals, a supposition which is not supported by the work of Morgan and Garrison (1) on dogs, in which on the alkaline diet parathyroid treatment produced markedly greater increases in serum calcium and in susceptibility to overdosage phenomena than did the neutral or acid diet. Bodansky, Blair and Jaffe report the diet which they used only in the most general terms and it is impossible to judge what its metabolic reaction may have been. No hydrogen ion concentration tests were made upon blood or urine. It is possible that the effect of fasting noted was that of a high phosphorus concentration in the serum rather than of change in reaction of the tissues. The lack of similar effect in fasted adult animals argues in favor of this

explanation. Bodansky and Jaffe (4) describe experiments with growing dogs in which at first normal response to parathyroid extract resulted but on continued treatment the serum calcium levels dropped to normal or lower than normal levels. Again it is difficult to discern whether any of the dogs received other than a low calcium–high phosphorus diet. They state that when the calcium reserves were replenished or the parathyroid dosage increased the usual rise in serum calcium resulted. This appears to point to the dependence of such rise upon the presence of a certain mobile calcium reserve.

Their later communication (5) contains details concerning four young dogs fed a basal diet to which various additions of calcium lactate were made. The response of these puppies to parathyroid injection is essentially like that found in the experiments here presented. Again, however, the "tolerance" resulting from long-continued parathyroid treatment reported by clinical observers (2, 6) is ascribed to some mechanism of compensation rather than to immunity, as thought by the latter. But the indispensability of the presence of an available reserve of calcium would appear to be a simpler explanation of this phenomenon. The ostitis fibrosa found in young dogs fed a calcium-deficient diet and kept in a state of chronic hyperparathyroidism is thought by Bodansky and Jaffe to be due primarily to the parathyroid treatment and to be distinct from the bone condition resulting from the calcium-low diet alone. It is interesting to observe in this connection that Becks and Weber (7) using tissues of dogs reared in this laboratory and on diets practically identical with the low-calcium diet used in a series of parathyroid injection experiments to be reported later, found typical ostitis fibrosa although no parathyroid injection had been made. The bony defects characteristic of this disease may indeed be secondary to the production and maintenance by any means for long periods in growing animals of abnormal serum calcium and inorganic phosphate values.

The contradictory conclusions of many recent workers concerning the relation of vitamin D to the parathyroid glands and the source of the excess serum calcium resulting from high dosage of irradiated ergosterol or of parathyroid extract may possibly be explained on the basis of widely varying intakes of calcium and phosphorus. Many of the studies were made with but little attention to the diet of the experimental animals.

The study here described was begun in order to attempt to find an answer to the questions which have recently arisen as to the mode of action of both vitamin D and parathyroid extract in producing their obvious effects upon serum calcium and phosphate as well as upon bone development and maintenance. In particular it was desired if possible to elucidate the rôle of the composition of the diets fed during administration of these agents in the response of the organism.

Plan of experiment. A series of experiments upon both dogs and rats was

planned, the diets being varied only as to calcium and phosphorus content and the vitamin D and parathyroid extract being administered to certain animals on each diet. Three types of calcium to phosphorus ratios were used, high, medium or normal and low. Only isolated food constituents were used in these diets which were alike except for the salt mixtures. Casein and wheat gluten were used as source of protein in all but two of the diets in which egg albumen was substituted for the casein. Only the high calcium-low phosphorus type of diet (table 1) was used in the experiments reported in this paper, although in at least one case (diet 20) the actual level of calcium was low. Salt mixture 6 has been previously described (1) and salt mixture 7 was made from 6 by addition of 108.4 parts of potassium dihydrogen phosphate in order to raise the phosphorus content of the diet to an approximately normal figure and to bring its calcium to phosphorus ratio close to 1. This mixture was used in the case of one group of dogs only, litter R.

The young dogs ate these diets readily, particularly those which were given the diets immediately on weaning. Whenever other food of any kind is given previously some difficulty may be experienced in training the dogs to eat the artificial mixture. These diets are all vitamin D-free and upon all of them bone deformities of varying degrees will develop unless some source of vitamin D is added. Even daylight and occasional sunning will not prevent rickets-like disease, particularly in the rapidly growing breeds if cod liver oil or viosterol[1] is not given. This statement applies to diets of normal calcium to phosphorus ratio and content almost as forcibly as to the other diets with abnormal calcium to phosphorus ratios. It is clear that dogs differ from rats in this respect, possibly through the adaptation of rats to the economical use of smaller amounts of light. The difference may even be connected with the observed discrepancy in the parathyroid apparatus of the two species, rats being relatively immune to the effect of any but very large doses of parathyroid hormone as well as of excessive amounts of irradiated ergosterol while dogs, like humans, are sensitive to such treatment.

It will be noted that no provision for the antiscorbutic vitamin is made in any of the diets described. In our first work with young dogs (8) lemon juice was included in all diets for this purpose but later this was discontinued in the belief that this species is able to synthesize the vitamin C. Since more than 100 young dogs have now been reared and maintained to the age of one year or more on vitamin C-free diets in this laboratory it appears

[1] Most of the parathyroid extract, cod liver oil and viosterol used in these experiments were kindly furnished by E. R. Squibb & Sons to whom we gladly express our gratitude. Each batch of these products was tested both by the Research Laboratory of E. R. Squibb & Sons and by us, the first named by administration to normal adult dogs and the other two by the line test on rats.

clear that dogs resemble rats and cattle in their freedom from the need of antiscorbutic provision. Several adult dogs have been kept longer than 2 years under similar conditions with no signs whatever of scurvy. The Yale group[2] have likewise found that vitamin C may be ignored in the diets provided for their dogs.

The amount of food (table 1) given young pups at weaning provides about 250 calories per kgm. per day. On smaller amounts the dogs do not grow satisfactorily and the quantity is seldom decreased to less than 200 calories per kgm. per day until the dogs have reached the age of five or six months. These animals are kept in confinement throughout the growing period. Naturally the large breeds continue to eat the full quota for a longer period than do the small, but even adult dogs seem to require close to 100 calories per kgm. per day for satisfactory maintenance. This is a larger amount than that advocated by Cowgill (9), but difference in type of dog as well as in caging, temperature and composition of diet may account for the discrepancy.

Procedure. Of the three litters here reported upon, two, P and R, were born in the laboratory and were placed at 5 to 7 weeks of age upon the synthetic diets. The other litter, X, was procured from the country and was not placed on the experimental diet until 7 weeks of age. It will be noted later that the behavior of the members of this litter exhibited differences from the others which may possibly be ascribed to this early period on mixed diet and natural outdoor life. The description of the dogs and their origin are given in table 2.

The routine treatment included x-ray records of the leg bones of all dogs at weaning and at four-weekly intervals thereafter, dental examinations at similar intervals, regular blood analyses both in animals given parathyroid extract and in their controls, calcium and phosphorus metabolic balances in certain representative cases, autopsies in all cases and preservation of parathyroid glands, jaws and femurs for histological and chemical examinations.

The dogs fed the high calcium-low phosphorus diets grew rapidly but early developed rachitic deformities. Tetany was never observed in these dogs nor did lameness or paralysis develop except occasionally in dogs 149 and 152. The animals given vitamin D with this diet were nearly normal.

Serum calcium and inorganic phosphate were determined by the methods previously described (1), all blood samples being drawn 14 to 16 hours after the injection of parathyroid extract whenever such treatment was indicated.

The serum calcium and inorganic phosphate. Nine young dogs of three litters were fed the high calcium-low phosphorus diets in this series, and

[2] Personal communication from Dr. Arthur H. Smith.

seven of these were later given parathyroid extract. As shown in charts 1 and 2, the dogs fed diets 21 and 22 with irradiated ergosterol or cod liver oil, nos. 147 and 154, responded to even small doses of parathyroid extract with noticeable increases in serum calcium. The order of increase was found to be maintained in dog 154 when the injections were continued over a period of 60 days. The same was true of dog 147, although the record here given covers only two series of injections, the earlier responses on diet 20 having been reported before (1). The litter mates of these animals, 144, 149, and 152, which were not given vitamin D showed only slight or entirely negative changes in serum calcium when similar doses were given. This confirms our previous findings on litters K and L (1).

Dogs 180 and 182 of litter X although showing the typical bone deformities produced by this diet without vitamin D responded to parathyroid treatment as though D were being given. Moreover, by x-ray examination[3]

TABLE 1

Composition of diets

Grams per kilogram per day

NUMBER OF DIET	CASEIN	EGG ALBUMIN	WHEAT GLUTEN	SUCROSE	AGAR	SALTS		LARD	BUTTER	YEAST	Ca	P	$\frac{Ca}{P}$
						Number of Hill	Amount				*per cent*	*per cent*	
20		5.0	15.0	21.3	0.4	6	1.0	2.4	3.6	0.6	0.23	0.14	1.6
21		5.0	15.0	21.3	0.4	6	3.0	2.4	3.6	0.6	0.74	0.09	8.2
22	5.0		15.0	21.3	0.4	6	3.0	2.4	3.6	0.6	0.65	0.13	5.0
23	5.0		15.0	21.3	0.4	7	3.0	2.4	3.6	0.6	0.75	0.91	0.8

these two dogs appeared to have benefited by the parathyroid treatment sufficiently to show "healed rickets" on autopsy, although their control, 179, without vitamin D or parathyroid, evidenced no healing. Figure 1, containing postmortem radiographs, shows plainly the definite healing which took place in 180 and 182, the parathyroid treated animals. As has been noted before, this litter came from the country where they had lived an outdoor life until seven weeks of age. It is conceivable that in spite of the abnormalities observed in their bony development a sufficient store of vitamin D and of calcium remained in their tissues to insure normal response to the parathyroid treatment and subsequent normal calci-

[3] Dr. E. S. Heald, roentgenologist at Alta Bates Hospital, Berkeley, California, kindly supplied us with an interpretation of all the radiographs obtained upon the animals used in this study. We are also indebted to the University of California Infirmary for the use of the Roentgen apparatus.

fication. Moreover, their serum calcium and phosphate figures when uninfluenced by parathyroid resembled those of their litter mate 181 which had cod liver oil as much as they did those of 179, without vitamin D. Healing at least to the same degree had not been seen in other animals on the same diet given similar parathyroid treatment without vitamin D and without normal response to the hormone injection although Bischoff (10) found improved calcium retention in rachitic dogs under these conditions. Moreover, in rats (11) definite healing as indicated by the line test and rise in serum phosphate was seen in all cases where parathyroid extract was administered. The very high calcium to phosphorus ratio, 8.2:1, of the diet used for litter X may well have some bearing upon the unusually high serum calcium values obtained.

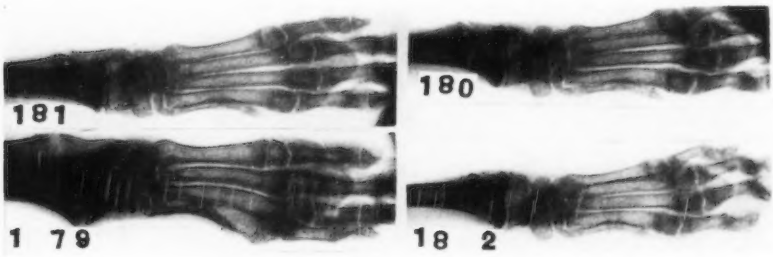

Fig. 1. Post-mortem radiographs of wrists of dogs of litter X on high calcium-low phosphorus diet, 181 with cod liver oil and 179 without source of vitamin D. No parathyroid extract was given these animals. Dogs 180 and 182 received no source of vitamin D but 180 was given 620 and 182 440 units of parathyroid extract. Note definite healing as compared with 179.

In an earlier series (8) of experiments, two litters of setters, one born in the laboratory, the other in the country, but of the same parentage, were also found to exhibit striking differences in amount of damage to bone development due to lack of vitamin D in the diet. The members of the litter which was born following a normal outdoor life of the mother exhibited on identical dietary régime in all but one case far more normal calcification than did those which were born following a pregnancy period passed by the mother in the laboratory.

Litter R after the first 26 days of study but after 117 days on the diet, was given enough phosphorus to make the diet practically normal. In addition dog 152 was given 1 gram cod liver oil per kgm. per day thus making its diet the same as that given 154. Dog 149 was continued without cod liver oil. Healing took place rapidly on this diet, no. 23, in both 149 and 152 although serum inorganic phosphorus remained low in 149 and

rose to the same level as that found in the control in 152. The control dog 154, however, had likewise shown a low serum phosphorus on the original diet, no. 22, although no evidences of bone abnormality were observable. On raising the phosphorus level in the diet the serum phosphorus in this animal rose to 5 or 6 mgm. per cent. This is the converse of the recent finding of Hess, Weinstock, Rivkin and Gross (12) that a rise in blood inorganic phosphorus may take place without healing of rickets.

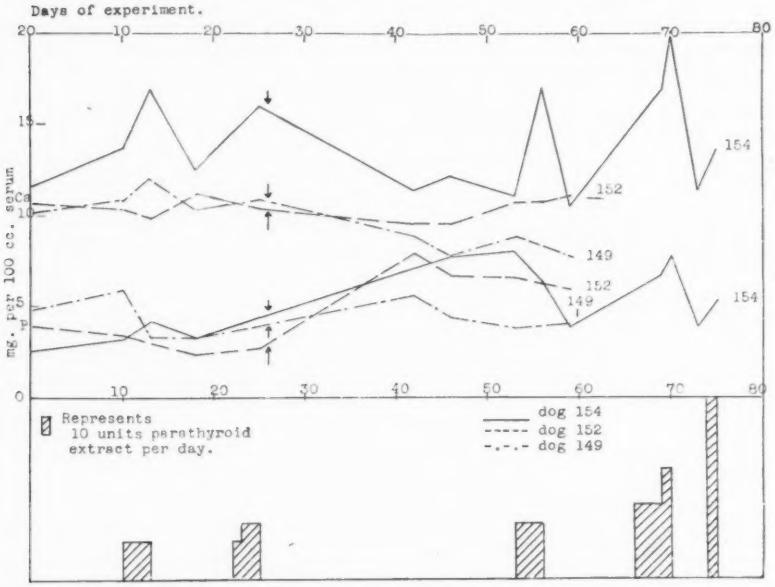

Chart 1. Serum Ca and inorganic P of 3 young dogs of litter R on high Ca-low P diet as affected by parathyroid dosage.

Dog 149 received no source of vitamin D, dog 152 received none until the date marked by the arrow after which it received cod liver oil as for 154. Dog 154 received 1 gram per kgm. of potent cod liver oil daily throughout the experiment. At the date marked by the arrow the phosphorus content of the diet of all 3 was increased from 0.2 to 0.9 per cent.

Note undiminished response of 154 to repeated parathyroid treatment as well as constant lack of response by 149 and 152.

The rise in inorganic phosphate in 152 and in 154 parallels the results obtained by Salvesen, Hastings and McIntosh (13) on oral administration of relatively large amounts of phosphate to so-called normal dogs. The resulting violent tetany which they observed occurred only when the serum calcium was depressed at the same time that the serum phosphate was increased. The amounts of phosphates added to the diet of our dogs (100

TABLE 2

Description of dogs and result of treatment

LITTER	NUMBER OF DOG	SEX	DIET	PARATHYROID EXTRACT INJECTION		WEIGHT		REMARKS
				Age when first given	Total units given	At first serum analysis	Final	
				days		*kgm.*	*kgm.*	
P	144	Male	21	233	270	6.5	7.6	Born in laboratory, placed on diet 20 at 38 days of age, changed to diet 21 at 230 days. Deformed and decalcified bones at autopsy
P	147	Male (With viosterol)	21	233	60	4.8	5.3	Decalcified but fairly straight bones. "Healed rickets"
R	149	Male	22 and 23	148	225	3.1	4.6	Born in laboratory, placed on diet 22 at 48 days of age, changed to diet 23 at 165 days. Extremely deformed, unable to stand during later stages of experiment. Decalcified and deformed bones
R	152	Male	22 and 23	148	225	5.2	6.5	Similar to 149
R	154	Female (With cod liver oil)	22 and 23	148	550	3.3	4.7	Practically normal bones. Died of overdosage
X	179	Female	21	None given		2.0	6.2	Born in the country, placed on diet at 49 days of age. "Severe rickets, no healing"
X	180	Male	21	117	620	2.5	4.5	"Severe rickets," followed by healing on parathyroid treatment
X	181	Female (With cod liver oil)	21	None given		1.9	4.0	Practically normal bones
X	182	Female	21	117	440	2.4	3.1	"Severe rickets" followed by healing on parathyroid treatment

to 200 mgm. P per kgm. per day) are less than those found by Salvesen, Hastings and McIntosh to produce clinical symptoms. The significant difference, however, lies in the fact that our dogs which had cod liver oil,

152 and 154, showed less depression of serum calcium than did 149 without vitamin D. Likewise, the latter animal had no rise in serum phosphate although the two dogs receiving vitamin D clearly showed this rise. This confirms the frequently assumed function of vitamin D as a regulator of both serum calcium and phosphate within certain limits. Moreover, the drop in serum calcium in the case of dog 149 in spite of the continued low level of serum phosphate supports the view that rise in serum phosphate cannot be the sole cause of lowered serum calcium as a result of the precipitation of calcium phosphate. A similar but greater fall in serum calcium was seen by Skaar (14) in rachitic pups when disodium phosphate was added to the diet.

Karelitz and Shohl (15) observed rapid rise in serum phosphate and decrease in serum calcium in rachitic rats given added phosphate without change in vitamin or light conditions. This was accompanied by healing of the rickets. However, Shohl, Bennett, and Weed (16) found that similar addition of phosphate to the diet of non-rachitic rats receiving the high calcium-low phosphorus diet and vitamin D made no such change in the blood constituents which remained normal.

Attention is drawn to the parallel condition seen in dog 156 on low calcium-high phosphorus diet without vitamin D (17) which on administration of calcium lactate exhibited a rapid rise in serum calcium and an accompanying depression of serum phosphorus. When cod liver oil was added to this reinforced diet a rapid rise in serum inorganic phosphorus resulted with little change in calcium level. The depressing effect of an increase in either calcium or phosphorus intake upon the serum level of the other element appears thus to be alleviated by the presence of vitamin D.

The low inorganic phosphate and normal serum calcium of dog 147 are similar to the figures found for dog 154 on similar diet with cod liver oil. The low serum calcium and moderately low phosphate of dog 144 without vitamin D are again parallel with similar figures for dogs 149 and 152 on diet 22. Apparently the disturbance of metabolism produced by the high calcium-low phosphorus diets used in these experiments is shown in low serum phosphate even when vitamin D is present and in reduced serum calcium as well when the vitamin is lacking. Such an effect is not observable during the early period of feeding (1) since high serum calcium and low phosphate are found without D in both dogs and rats. As seen in chart 1, however, a gradual fall in serum calcium occurs as the diet is continued. When vitamin D is present the only change which occurs is in the phosphate which tends to fall.

As is shown in chart 2, a rise in the Ca:P ratio of the diet and of its calcium content appeared to decrease the hypercalcemia induced by parathyroid extract in dog 144 which received no vitamin D but to make little change in the response of the dog which received viosterol. This was

accompanied by a rise in the serum phosphorus in dog 144. Thus the response to parathyroid treatment by these two dogs became more like that of litter R on diet 22, also of high Ca:P ratio, when they were placed on diet 21.

Ample confirmation of our finding that vitamin D intensifies the effects of parathyroid extract has been furnished by the work of Abeloff and Sobel (18) and of Johnson (19). In the former study with guinea pigs no dietary control was attempted but repeated injections of the parathyroid preparation produced the bone lesions of generalized fibrous osteitis which were

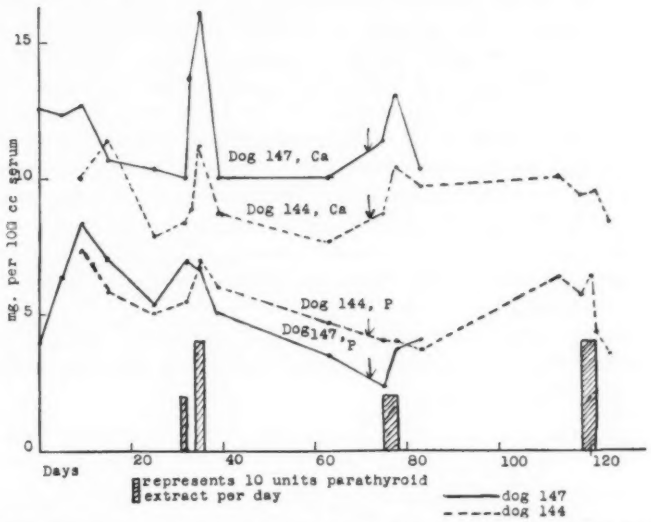

Chart 2. Serum Ca and inorganic P of 2 young dogs of litter P on low Ca-low P and high Ca-low P diets as affected by parathyroid dosage.

Dog 144 received no source of vitamin D, dog 147 received 5 mgm. viosterol (100 D) daily. At the date marked by the arrow, the Ca content of the diet of both was increased from 0.23 to 0.74 per cent.

Note exaggerated response to parathyroid treatment by the dog which received the viosterol.

not prevented by simultaneous administration of viosterol. Johnson (19) fed most of the young dogs given parathormone a low calcium diet of lean meat, bread, orange juice and cod liver oil and a diet of normal calcium and phosphorus content to the rats. Osteitis fibrosa was produced in all cases by continued parathormone treatment which was more severe when viosterol was given in addition. Metastatic calcification in the kidneys in the latter case was more marked than when the parathormone was given alone.

SUMMARY. 1. On high calcium-low phosphorus synthetic diets marked hypercalcemia resulted in young dogs from even moderate doses of parathyroid extract when vitamin D (cod liver oil or irradiated ergosterol) was given. When no vitamin D was given there was little change in serum calcium and no other obvious result of the parathyroid treatment except that a rise in serum inorganic phosphorus occurred in all cases both with and without vitamin D.

2. Two young animals on a diet of very high calcium to phosphorus ratio without vitamin D responded with hypercalcemia to parathyroid extract even though normal bone development had not been obtained. This may be due to the presence of sufficient stores of the vitamin and of calcium reserve from early intake and sun exposure. Healing of rickets occurred in these dogs as a result of the parathyroid treatment.

3. Low serum calcium and moderately low serum phosphate were found in dogs without vitamin D on high calcium-low phosphorus intake but normal or high serum calcium and low serum phosphate in those receiving vitamin D. Addition of phosphate to the diet raised the serum phosphate in dogs with vitamin D but produced no change except further lowering of the calcium in the animal without vitamin D. No change in response to parathyroid was brought about by this change in diet either with or without vitamin D.

4. Parallel addition of calcium to a low calcium-low phosphorus diet resulted in decreased response to parathyroid extract injection particularly in the dog without vitamin D.

CONCLUSIONS

1. The source of increased serum calcium seen in dogs on diets of high calcium to phosphorus ratio with vitamin D when parathyroid extract is administered in moderate doses may be the calcium of the food or of an easily mobilized reserve, rather than of the essential tissues. When vitamin D is not given the food or reserve calcium is not usually so readily available and the hypercalcemia is of lower magnitude.

2. There is some evidence of healing of rachitic lesions when animals on diets of high Ca:P ratio without vitamin D are given parathyroid extract. An increased circulation of inorganic phosphate either from the intestine or the soft tissues stimulated by the hormone would account for this effect. This is in contrast with the usually observed fall in inorganic serum phosphate following parathyroid treatment.

3. The level of serum inorganic phosphate of dogs is governed not only by the level of phosphorus in the diet but also by the Ca:P ratio whether vitamin D is present or not but the serum calcium level tends to respond more slowly to changes in dietary calcium.

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THE EFFECT OF DIET ON RESPONSE TO PARATHYROID EXTRACT AND VITAMIN D

III. The Effect of Low Calcium-High Phosphorus Diets in Dogs

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In pursuance of the investigation of the effect of the calcium and phosphorus content and calcium to phosphorus ratio of the diet on response to parathyroid extract in the presence and absence of vitamin D a group of young dogs was fed a low calcium-high phosphorus diet, some being given cod liver oil in addition and representatives of both D-rich and D-free groups later being subjected to the influence of parathyroid extract. This diet was designated number 24, and is made up as follows, the figures representing grams per kilogram of body weight per day: casein 5, wheat gluten 15, sucrose 21.3, agar 0.5, low calcium salt mixture 4 (1) 3, lard 2.4, butter 3.6, yeast 0.6. This mixture has the unusually low calcium to phosphorus ratio of 0.1 and contains 0.10 per cent calcium and 1.02 per cent phosphorus.

The procedure and methods have been described previously (1). Our object in this study was not only the securing of data as to blood and metabolism changes produced by vitamin D and by parathyroid extract in dogs fed a low calcium-high phosphorus diet but also the comparison of these effects in small and large types of dogs in the hope of correlating the data with rate of growth.

Thirteen dogs of 4 litters were placed on this diet at 4 to 5 weeks of age and were kept on it throughout the experimental period of 10 to 16 weeks. The description and sources of these animals are given in table 1. It will be noted that 9 of the dogs (litters S, W, Y) were of a small breed and 4 (litter Z) of a rapidly growing large breed. The latter dogs evidenced more acute and ealier symptoms of the calcium deficit than did the small terriers of the other litters. Even the animals which received cod liver oil were deformed and stunted although not to the extreme degree manifested by those without vitamin D. Tetany and loss of muscular control occurred in nearly all cases after about 50 to 70 days on the diet. Soreness of the joints, hypotonia and inability to stand developed in all the animals without vitamin D and in most marked form in the large dogs, 191 and 192. These symptoms are strikingly similar to those described in the human cases of hyperparathyroidism described by Wilder (2) and by Barr, Bulger and Dixon (3)

and by Bodansky and Jaffe (4) in dogs. The dogs which received parathyroid extract showed little if any more exaggerated effects of the diet than did those which were not given the parathyroid treatment. The tissues of these animals were not examined histologically but their condition and the diet given them were nearly identical with those of an earlier series of animals produced in this laboratory which have been reported by Becks and Weber (5) as showing typical osteitis fibrosa.

Low calcium diets have frequently been fed in order to observe the effect upon bone structure. Dibbelt (6), Aron and Sebauer (7) and Mellanby (8) are some of those who found low bone ash on such diets and the struc-

TABLE 1

Description of dogs

LITTER	NUMBER OF DOG	SEX	DATE OF BIRTH	BREED	REMARKS
S	156	♂	August 5, 1929	Small fox terrier	Born in laboratory, weaned September 20 to diet 24, low Ca-high P
	157	♂			
	158	♂			
	159	♂			
W	174	♂	January 10, 1930	Small fox terrier	Brought to laboratory February 5, 1930, placed at once on diet 24
	175	♂			
	176	♀			
	177	♀			
Y	184	♀	January 24, 1930	Small fox terrier	Brought to laboratory March 6, placed at once on diet 24
Z	187	♀	January 30, 1930	German shepherd	Brought to laboratory March 6, 1930, placed at once on diet 24
	188	♂			
	191	♂			
	192	♂			

ture characteristic of osteoporosis. But in all these studies mixtures of natural foods were used and no attention was paid to light conditions. It is questionable whether any of the diets were either adequately supplied with the fat soluble vitamins or entirely free from them. Consistently low serum calcium and high phosphate were not demonstrated in any case. Histological examination of the tissues of young dogs reared in this laboratory on purified diets low in calcium and free from vitamin D has recently indicated (Becks and Weber, 5) the production of typical osteitis fibrosa cystica (von Recklinghausen). It might be questioned whether the tissue changes seen by Bodanksy and Jaffe (4) followed from the hyperparathyroidism only rather than the hypocalcemia reported in

TABLE 2

Effect of parathyroid extract upon serum calcium and inorganic phosphorus of young dogs on low calcium-high phosphorus intake

LITTER	DOG	VITAMIN D	PARATHYROID DOSAGE	DATES (1929-30)	WEIGHT	SERUM ANALYSIS		REMARKS
						Ca	P	
			units per day		*kgm.*	*mgm. per 100 cc.*	*mgm. per 100 cc.*	
S	156	None		Dec. 10	2.0	6.5	3.7	Tetany, complete loss of use of hind quarters on 83rd day on the diet. "Severe rickets"
				Dec. 11–13				2 gm. calcium lactate given daily
				Dec. 14–18	2.0	10.4	2.2	No tetany, rapid recovery of activity
		1 gm. CLO per kgm. per day		Dec. 19–Feb. 3	2.0–1.8	10.5	6.6	1 gm. per kgm. per day cod liver oil given in addition to 2 gm. calcium lactate and basal diet. Normal walk
S	157	1 gm. CLO per kgm. per day		Dec. 10	3.3	9.3	7.6	2 analyses. Some flaring of epiphyses, most nearly normal of the litter
			30	Dec. 13	3.7	10.5	8.4	Symptoms of overdosage
			None	Jan. 3–13		10.1	7.3	3 analyses
			30	Jan. 14–17		11.0	7.4	
			60	Feb. 3–6	3.8	10.8	6.2	Occasional tetany. Cries at touch, lame from 141st day on the diet
			80	Feb. 11				
			100	Feb. 18		21.3	10.9	Symptoms of overdosage. Practically normal bones on autopsy
S	158	None	None	Dec. 10	2.1	8.2	4.1	Tetany, paralysis of hind quarters from 63rd day on the diet
			30	Dec. 11		9.5	3.3	Died in tetanic spasm. "Severe active rickets"

S	159	None		Dec. 10	2.0	7.8	3.2	Tetany, paralysis of hind quarters from 67th day on the diet
			30	Dec. 10–14		12.1	3.3	"Severe active rickets"
			30	Dec. 13–17		8.4	2.7	No overdosage symptoms
W	174	1 gm. CLO per kgm. per day		Mar. 13	1.5	11.8	6.7	Normal
				Apr. 7	2.0			Died of lung infection
W	176	1 gm. CLO per kgm. per day		Mar. 13–June 2	1.4–3.2	10.0	6.1	4 analyses. Normal activity
			30	Apr. 30–May 2	2.7	13.7	6.5	Nearly normal epiphyses
			30	May 5–8		10.2	7.7	
			50	May 12–15	3.1	10.2	6.0	Active, normal shaped legs
			50	May 18–22		10.8	5.6	
			50	May 27				
			70	May 28				
			100	May 29	3.2	15.8	4.8	Normal calcification, much fat
W	177	None		Mar. 13–June 2	1.1–2.8	9.5	5.4	Normal activity
			30	Apr. 30–May 2	2.4	11.2	7.4	Deformed forelegs
			30	May 5–8		8.1	6.2	
			50	May 12–15	2.6	7.6	5.1	
			50	May 18–22		8.9	5.4	
			50	May 27				Most abnormal ribs and epiphyses of the litter found on autopsy
			70	May 28				
			100	May 29	2.8	12.0	3.8	No symptoms of overdosage
W	175	None		Mar. 13–May 2	1.4	9.9	5.2	Lame and tetanic from 32nd day on the diet
			30	May 1				
			30	May 2	1.6	12.2	3.8	Blind and deaf. Paralyzed in hind quarters from 75th day
Y	184	None		Mar. 27–May 2	1.7–2.4	10.1	5.5	Normal activity
		None		May 15–June 2	2.7–3.0	8.8	4.8	Tetany, lame

TABLE 2—*Concluded*

LITTER	DOG	VITAMIN D	PARATHYROID DOSAGE	DATES (1929–30)	WEIGHT	SERUM ANALYSIS		REMARKS
						Ca	P	
			units per day		*kgm.*	*mgm. per 100 cc.*	*mgm. per 100 cc.*	
Z	187	2 gm. CLO per kgm. per day		Mar. 20–May 2	2.9–6.4	10.8	6.9	
			30	May 9–12	7.4	12.3	7.0	Cries at touch
			30	May 15–25	7.0	12.4	4.7	Lame
			50	May 24–28		11.7	6.9	Unable to stand from 72nd day
			50	June 3				
			70	June 4				
			100	June 5	7.7	16.0	6.6	Bones found soft and hemorrhagic on autopsy
Z	188	2 gm. CLO per kgm. per day		Apr. 16–June 5	4.8–9.4	11.3	7.5	Lame, hind quarters paralyzed from 72nd day. Extremely bowed legs and flat feet
Z	191	None		Mar. 20–June 5	2.6–7.1	9.7	5.0	Extremely deformed legs. Tetany, paralyzed hind quarters from 64th day. Cries at touch. At autopsy bones found soft and hemorrhagic
Z	192	None		Apr. 16–May 2	4.7–5.5	9.8	5.4	
			30	May 9–12	5.6	9.4	3.7	Extremely deformed. Unable to stand. Cries at touch
			30	May 15–25	6.8	10.4	5.0	Unable to stand from 52nd day. Tetany first noted on 52nd day
			50	May 24–28		9.8	3.3	
			50	June 3				
			70	June 4				
			100	June 5	6.0	10.8	2.7	Frequent tetanic spasms. Bones found soft and spongy on autopsy, moderate amount of fat

their young dogs. Indeed in a later experiment Jaffe, Bodansky and Chandler (20) found marrow fibrosis in a series of young dogs on low calcium diet without parathyroid treatment. It seems probable that osteitis fibrosa was present in our dogs reared on the low calcium diet without vitamin D as described in this report. In 3 of the 5 dogs given cod liver oil with the low calcium diet tetany, lameness and depression eventually developed. In 7 of the 8 dogs which did not receive vitamin D additions similar symptoms but of more severe grade appeared. The three exceptions 174, 176 and 177 were terriers of a very small type belonging to one litter. One of these, 174, died of a lung infection after only 54 days on the diet. As may be noted in table 2 the muscular paralysis occurred in the dogs without cod liver oil from the 32nd to the 83rd day on the diet, and in the dogs which received cod liver oil, on the 72nd in two and on the 141st day in the third. The former 2 were large shepherd dogs, the latter a small fox terrier. This illustrates a point which has been noted by others, that the large rapidly growing animal suffers more severely from a calcium or phosphorus deficit than does the slow growing animal of smaller type.

The pain in muscles and bones shown by the dogs of litter Z, extreme deformity of legs and spine, inability to stand and frequent tetany were particularly striking. One of the small dogs, 175, was blind and deaf after a few weeks on the diet and helpless from the 75th day. Low serum calcium and moderately low phosphate characterized the dogs without cod liver oil, but normal serum calcium and phosphate were maintained by those which received cod liver oil.[1]

Calcium lactate was given to dog 156 (table 2) when helpless and tetanic on the 127th day of its life, with immediate rise in serum calcium and rapid recovery of muscular control but without rise in serum phosphate. After nine days cod liver oil was also given with immediate rise in serum phosphate. This animal remained normal during the rest of the experiment. This conduct parallels the difference in serum composition shown by dogs 149 and 152 (9) on high calcium diet when phosphate was added to the diet of both, but cod liver oil to that of 152 only.

The serum calcium and inorganic phosphate. Parathyroid extract produced but little rise in serum calcium in dogs 157, 176 and 187 which were receiving cod liver oil, except for the first dose and the final greatly increased dose. Similarly dogs 159, 177 and 192 which received no source of vitamin D responded only to the first dose if at all. This behavior is illustrated in chart 1 by serum calcium curves for dogs 176 and 177. This might be explained as due to the early exhaustion of mobile calcium reserves, a condition paralleled by the "immunity" to parathyroid treatment seen by Albright, Bauer, Ropes and Aub (10) in the treatment of clinical

[1] We are indebted to E. R. Squibb & Sons for most of the parathyroid extract and cod liver oil used in this study.

cases with the hormone. They obtained a typical low calcium response in most of their cases, all but one of which were on low calcium intake. The one case in which calcium lactate was given showed the high-calcium response, that is, immediate and continued exaggerated hypercalcemia, as contrasted with the small and decreasing responses in most of the other cases. The fall in serum phosphate on parathyroid injection followed by a rise which they obtained in nearly all cases is also typical of the low-cal-

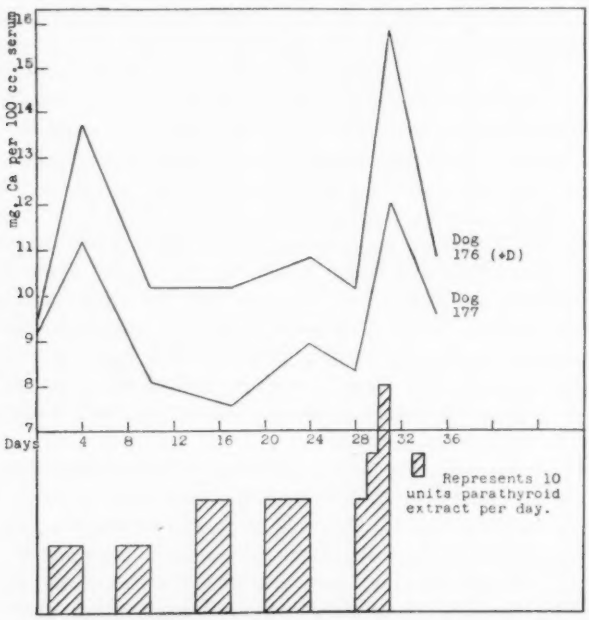

Chart 1. Serum Ca of 2 young dogs of litter W on low Ca-high P diet as affected by parathyroid dosage.

Dog 176 received 2 grams of potent cod liver oil per kgm. daily, dog 177 no source of vitamin D.

Note preliminary rise in serum Ca on parathyroid treatment followed by lack of response until a very large dose is given.

cium response, particularly when no vitamin D is present. On high calcium-low phosphorus intake on the other hand a rise in phosphate is likely to occur especially with vitamin D present. These investigators declare that the decreasing hypercalcemia of continued parathyroid treatment cannot be due to exhaustion of calcium reserves because ammonium chloride ingestion in such cases can still produce an increased calcium elimination. But the elimination thus secured may be produced by a different mech-

anism, perhaps by solution of the bony cortex as well as of the reserve trabeculae. They found that the calcium and phosphorus loss resulting from ammonium chloride administration occurred immediately and simultaneously whereas that following parathyroid injection occurred gradually and with considerable lag in the case of the calcium but abruptly in the case of phosphorus. Moreover, the loss of these elements was in the well-known bone ratio in the case of ammonium chloride administration, but was irregular in the case of the parathormone effect.

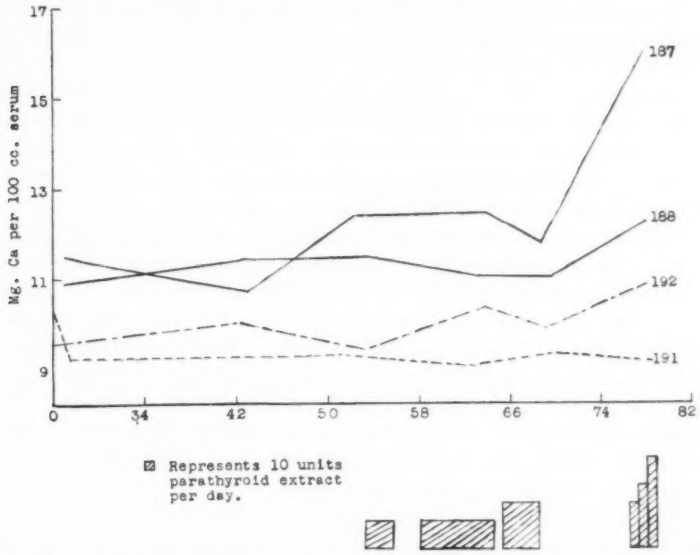

Chart 2. Serum calcium of 4 young dogs of litter Z on low calcium-high phosphorus diet as affected by parathyroid dosage.

Dogs 187 and 188 were given 2 grams potent cod liver oil per kgm. daily, dogs 191 and 192 receiving no vitamin D.

Dogs 188 and 191 were not given parathyroid extract.

Note moderate response to parathyroid treatment by 187 and reduced response by 192.

The work of Percival and Stewart (11) on subjects with low serum calcium and presumably low calcium reserves tends to confirm the assumption that the parathyroid hormone mobilizes these reserves. They found it difficult to produce hypercalcemias except for short periods and by massive doses in such cases. These findings parallel ours as shown in table 2. The dogs which received cod liver oil apparently were able to make small calcium reserve deposits even while parathyroid treatment was continued only to throw these into the serum when large doses, 50, 70, and 100 units

on each of three successive days, were given (dogs 157, 176, 187). Those without vitamin D showed much less hypercalcemia when the final large doses were given (dogs 177 and 192). This latter fact would seem to argue against the mobilization of bone cortex material by parathyroid action even under these rather drastic conditions.

It is obvious, however, that the dogs on this diet without vitamin D show hypercalcemias on administration of the initial dose of parathyroid extract which are nearly as marked as do those with vitamin D. Thus in litter S, 158 had an increase of 1.3 mgm. calcium per 100 cc. serum following 30 units dosage and 159 of 4.3, as compared with 1.2 in 157, which received cod liver oil. In litter W, there were increases of 2.5 and 2.0 in the dogs without vitamin D and of 4.2 in the dog which received cod liver oil (chart 1). In litter Z, however, dog 192 without cod liver oil showed no rise in serum calcium until 100 units were given, while 187, with cod liver oil had a rise of 1.5 after the first series of doses of 30 units (chart 2). These findings differ from those observed in dogs and rats on high calcium diets but are paralleled by our experiments on rats on low calcium—high phosphorus diet (11). As in the latter study the inorganic phosphorus of the serum of these dogs tends to be depressed by the parathyroid hormone.

It is difficult to reconcile these changes in serum phosphate, however, with the following or accompanying serum calcium rises unless there are entirely separate mechanisms for the effects on calcium and phosphate or unless the greatly increased level of serum phosphate stimulates urinary excretion sufficiently to account for the rapid lowering of that level and consequent possible rise in calcium concentration. The reported diuretic action of the hormone is in line with this possibility (15). Albright, Bauer, Ropes, and Aub (10) clearly saw the primary effect on phosphorus metabolism, but because their cases were nearly all on low calcium diet assumed that the effect is always that of lowering the serum phosphate. Tsai and Hsu (16) in an interesting study used four dogs in which the serum calcium and phosphate were determined at frequent intervals in the 24 hours after intravenous injection of parathormone. In three of these dogs consistent increases in serum inorganic phosphate were seen but in the fourth a marked lowering of this value. Nothing is said as to the habitual diet of these animals but both the preliminary blood findings and the effect of the hormone indicate that the three in which the inorganic phosphorus was raised were probably previously on high or normal calcium-low phosphorus with vitamin D, while the fourth, in which the phosphate was lowered, may have been on low calcium-high phosphorus diet. The authors treat the effect seen in the fourth animal as an exception and draw the probably erroneous conclusion that the maximum rises of calcium and phosphorus bear the bone ratio of 2 and that the excess calcium is therefore of bone origin. Thus Albright, Bauer, Ropes, and Aub assume that serum phos-

phate is always lowered, whereas Tsai and Hsu assume that it is always raised. Actually the effect appears to be governed by the preliminary level of serum phosphate which in turn is largely governed by the calcium-phosphorus level and ratio of the diet.

It is impossible to judge whether the same explanation can be offered for the initial hypercalcemia of the dogs without vitamin D as was suggested for rats on a similar diet (12). Inspection of the tibiae of the rats

TABLE 3

Ca and P exchange on low Ca-high P intake as affected by parathyroid extract

Dogs 187, 191 and 192 were members of litter Z born January 30, 1930. Dog 187 received 2 grams potent cod liver oil per kgm. per day. Dogs 191 and 192 received no vitamin D.

All figures are in milligrams per kilogram per day.

DOG	PERIOD	DATES (1930)	WEIGHT	PARATHYROID DOSAGE	INTAKE		OUTPUT				BALANCES		PER CENT OF INTAKE RETAINED	
							Ca		P					
					Ca	P	Urine	Feces	Urine	Feces	Ca	P	Ca	P
			kgm.	*units per day*										
187	1	April 16–April 23	5.2–5.9		35	342	2	3	277	31	30	34	85	8
	2	April 24–April 30	5.9–6.4		35	342	2	4	257	43	29	42	82	12
	3	May 8–14	7.1–7.4	15*	31	325	2	2	235	25	27	65	87	20
	4	May 15–23	7.4–7.0	30	21	218	1	4	183	31	16	4	79	2
191	1	April 16–April 23	4.6–4.7		41	398	2	25	345	45	14	8	34	2
	2	April 26–May 2	5.2–5.7		36	342	2	18	274	36	16	32	44	9
	3	May 8–23	6.1–7.1		30	303	2	19	232	42	9	29	30	2
192	1	April 16–April 23	4.6–4.7		28	263	3	20	174	49	5	40	17	15
	2	April 26–May 2	4.8–4.8		32	322	3	31	230	62	−2	30		9
	3	May 8–14	5.4–5.6	15*	34	325	3	29	224	56	2	45	6	13
	4	May 15–23	5.6–6.8	30	26	266	2	23	192	62	1	12	4	4

* Given in 3 doses of 30 units each on May 8–11.

on low calcium diet without vitamin D revealed a fairly large number of irregularly calcified trabeculae which were thought to represent sufficient calcium reserve to account for the rise in serum calcium observed. If the young dogs of terrier type of litters S and W were able to make such deposits on their restricted calcium intake the relatively normal initial rise in serum calcium might follow, whereas the large German shepherd dog, 192 of

litter Z, might be supposed to be able to accumulate less reserve due to more rapid growth and consequently to show little or no rise in serum calcium on parathyroid treatment. The low calcium retentions shown (table 3) by this animal argue in favor of this supposition. Its litter mate 187 receiving cod liver oil, showed small but fairly well sustained hypercalcemia on parathyroid treatment and small but well sustained calcium retentions.

Metabolic balances on low calcium-high phosphorus diets. The calcium and phosphorus metabolism of three dogs of litter Z on diet no. 24 was studied during three or four periods of 5 to 15 days each as shown in table 3. Dog 187 which received cod liver oil showed notably better calcium retention in the first two periods, without parathyroid, than did dogs 191 and 192, which received no vitamin D. The phosphorus retention in these periods in all three dogs was small particularly in comparison with intake. The administration of an average of 15 units of parathyroid extract per day during the third period made no consistent change in these retentions, but an average of 30 units daily in the fourth period decreased the phosphorus balance in both 187 and 192. The hormone was administered during the first part of the third period in order to provide for any lag in its effect upon excretion, but was given continuously throughout the fourth period.

On the whole the difference between balances in control as compared with parathyroid periods is not notable, but the better retention of calcium in the dog receiving cod liver oil as compared with those receiving no vitamin D is striking. This is in agreement with the statement of Harris and Innes (13) as to the primary function of vitamin D. Here again the large phosphorus losses in the urine and the low relative retentions of that element on a diet containing an excess thereof point to the predominating importance of the phosphorus metabolism in disturbances of bone development. This view has been expressed by Shohl and Bennett (14) on the basis of similar observations upon young dogs. Dog 192 showed definitely lower retention of calcium than did 191 but somewhat better phosphorus retention except in the fourth period where 30 units of parathyroid extract were given daily to 192 but none to 191. Dog 192 was plainly in greater distress and was more weakened and deformed by the diet throughout the experiment than was 191. This discrepancy developed before the parathyroid treatment began so that it is doubtful whether the greater severity of symptoms can be ascribed to this treatment. Dog 188 which received no parathyroid extract was throughout in worse condition than its control 187 which was given parathyroid treatment.

Only a small amount of calcium was excreted in the urine by these low calcium-fed dogs either with or without vitamin D and parathyroid extract. The calcium losses which occurred in the dogs without vitamin D were almost entirely from the intestine. The excess phosphorus was largely excreted in the urine in all cases and the per cent of total excretion thus elim-

inated was 80 to 90. Better phosphorus retention appears to be associated with lowered urinary phosphorus and better calcium retention with lowered fecal calcium. The conclusion of Orr, Holt, Boone, and Wilkins (17) that high phosphorus intake is followed by lowered calcium retention because of precipitation of insoluble calcium phosphate in the intestine applies here only to the dogs which did not receive cod liver oil. If vitamin D tends to prevent the re-excretion of calcium into the gut, possibly because of deposit in the bones, as certain studies appear to indicate (Bergeim, 18; Taylor and Weld, 19) the effect on fecal calcium noted in table 3 is to be expected. The calcium to phosphorus ratio of the feces of dog 187, receiving cod liver oil, was 0.1, identical with that of the diet, but those of the two dogs, 191 and 192, receiving no vitamin D, was 0.5 and 0.4, indicating a disproportionately large loss of dietary calcium as compared with the phosphorus excretion. Similarly elevated calcium to phosphorus fecal ratios were not seen in dogs on diets of normal Ca:P ratio without vitamin D (20).

In evaluating the results of the metabolism tests it must be borne in mind that only three dogs were used and that these were of the large rapidly growing type. Possibly effects of different magnitude or even direction might be observed in small dogs under the same conditions. The difference in magnitude of effect of the parathyroid extract on the serum values of the two types of dog is sufficient to warrant caution in applying the metabolism results to other types and species.

SUMMARY. 1. A low calcium-high phosphorus synthetic diet of calcium to phosphorus ratio 0.1 without vitamin D produced severe bone deformities, tetany, and muscular paralysis in 7 out of 8 young dogs. Even when supplemented by 1 or 2 grams of potent cod liver oil per kilogram per day this diet brought about similar but less severe symptoms in 3 out of 5 young dogs.

2. The dogs without cod liver oil had low serum calcium and low or normal inorganic phosphate but those given cod liver oil showed normal values.

3. The first doses of parathyroid extract (90 units in three days) stimulated slight hypercalcemia in all except in one large dog. Further administration, however, produced little or no effect unless the dose was greatly increased.

The rise in serum calcium was nearly the same in the small dogs whether vitamin D was present or not but was very low in the one large dog without vitamin D.

4. The addition of cod liver oil caused better retention of calcium in the one dog examined than was secured without it in two similar dogs. Parathyroid extract in doses of 30 units daily (about 4 units per kgm.) caused little change in calcium retention but lessened phosphorus retention in both the dog which received vitamin D and the dog which did not.

5. The ratio of calcium to phosphorus in the feces of the dog which received vitamin D is seen to be the same as that of the diet but is considerably higher in the feces of the two dogs which received no vitamin D.

CONCLUSIONS

1. The failure of parathyroid extract to produce continued hypercalcemia in animals fed low calcium diets is possibly due to the exhaustion of their limited calcium reserves and the lack of sufficient incoming calcium from the intestine. The further rise secured with massive dosage may be due either to the exploitation of slight reserves piled up even in spite of continued mild parathyroid treatment or to solution of bone, but the evidence appears better for the former explanation.

2. Vitamin D apparently acts so as to prevent re-excretion of absorbed calcium into the intestine, because of deposition in bone or elsewhere, rather than by increase in original absorption.

3. Parathyroid extract may release inorganic phosphate from organic compounds in the tissues or fluids and therefore tend to decrease phosphorus retention in animals in which the serum phosphate is already normal or high but may increase phosphorus retention in growing animals with previously low serum phosphate.

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EFFECT OF DIET ON RESPONSE TO PARATHYROID EXTRACT AND VITAMIN D

IV. The Effect of Diets of Normal Calcium and Phosphorus Content in Dogs

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In continuation of the study of the effect of the calcium and phosphorus content of the diet and of vitamin D upon the symptoms following the injection of parathyroid extract, sixteen young dogs of four litters were fed from weaning supposedly normal diets, nos. 26, 27, and 28 of Ca:P ratios 0.7, 1.4, and 1.0. The description of the diets is given in table 1 and of the dogs in table 2.

Two dogs, 185 and 190, of litter Z, were given increasing doses of parathyroid extract beginning on the one hundred and second day of life and continuing for twenty-two days. A total of 750 units of the extract was administered to each animal. Dog 185 which received cod liver oil responded with continuously rising serum calcium, but dog 190 which had no vitamin D showed no such rise. In table 3 it will be noted that all these dogs without as well as with vitamin D, without parathyroid treatment, had nearly the same concentrations of serum calcium and inorganic phosphorus, averaging 11.4 mgm. calcium and 6.0 mgm. phosphorus per 100 cc. of serum. None of the dogs of the large breeds, that is, litters Z, A1 and B1, were normal in appearance, however, nor as shown by x-ray examination of the bones. Indeed but little difference could be seen between those which received cod liver oil and those which did not, although the dog which received the cod liver oil, 186, showed distinctly better epiphyseal development than did its littermate, 189, without vitamin D. Apparently the very rapidly growing large breeds of dogs are as difficult to save from rachitic lesions as are similarly rapidly growing children. The development of obvious deformities, of ragged and cuffed epiphyses while the blood findings were normal is further confirmation of the suspicion that abnormal concentrations of calcium and phosphate in the serum may be parallel or following phenomena rather than the prime cause of failure of ossification.

The small dogs of litter V on the other hand were practically normal on this diet, particularly dog 170 which received cod liver oil.

All of the dogs of litters A1 and B1 were given one to three injections of parathyroid extract in doses of 100 units each, between the sixth and eighth months of their lives. These two litters were of the same type and were used chiefly to note the effect of the addition of acid and alkali to the diet upon response to the parathyroid hormone. Ammonium chloride was given dogs 204 and 206, and sodium carbonate to dogs 201, 205, and 202 in the amounts of 0.6 and 0.8 gram per kgm. per day respectively. The diet of dogs 207, 210, 212, and 214 was kept neutral. All of these animals except nos. 210, 214, and 201 were given 2 grams cod liver oil per kgm. per day as source of vitamin D. No difference due to the absence of the cod liver oil could be seen in the response of these three dogs at six to

TABLE 1

Composition of diets

Grams per kilogram per day

NUMBER OF DIET	CASEIN	WHEAT GLUTEN	SUCROSE	AGAR	SALTS		LARD	BUTTER	YEAST	Ca	P	$\frac{Ca}{P}$
					Number of mix	Amount				*per cent*	*per cent*	
22	5.0	15.0	21.3	0.4	6	3.0	2.4	3.6	0.6	0.65	0.13	5.0
24	5.0	15.0	21.3	0.4	4a	3.0	2.4	3.6	0.6	0.10	1.02	0.1
25	5.0	15.0	21.3	0.4	5	1.5	2.4	3.6	0.6	0.52	0.42	1.2
26	5.0	15.0	21.3	0.4	5	3.0	2.4	3.6	0.6	0.80	0.57	1.4
27	5.0	15.0	21.3	0.4	185	1.5	2.4	3.6	0.6	0.36	0.49	0.7
28	19.2		21.3	0.4	5	1.0	2.4	3.6	0.6	0.47	0.48	1.0

nine months of age to the administration of the hormone as compared with that shown by the other six dogs of these two litters. This is in contrast with the lack of hypercalcemia exhibited by dog 190 when so treated at a considerably earlier age, when three to four months old. The difference may be ascribed to a superior store of calcium reserves in the older dogs or to the smaller demands for the element due to their decreased rate of growth or to both these interlocking factors. A similar increase in parathyroid hypercalcemia with age was not seen, however, in the dogs on high calcium-low phosphorus diet without vitamin D (1), which were tested from the third to the eighth months of their lives. The determining factors appear to be the Ca:P ratio of the diet as well as the age and rate of growth. Thus rats on diets (2) of normal Ca:P ratio without vitamin D showed nearly as large increases in serum calcium on injection of the parathyroid extract as did those with vitamin D. Also, as shown previously (3), both

dogs and rats on diets of low Ca:P ratio respond to the first injection of the extract with even higher rises in serum calcium when no vitamin is given than when the vitamin is present in the diet. However, the maximum level attained in the former group is still usually lower than the normal serum-calcium value of these animals.

TABLE 2

Description of dogs

LITTER	NUMBER OF DOG	SEX	DATE OF BIRTH	BREED	REMARKS
U	163	Female	Nov. 10, 1929	Airedale	Brought to laboratory Jan. 31, 1930. All were in poor state of nutrition, placed at once in pairs on diets 22, 24 and 25
	164	Female			
	165	Female			
	166	Female			
	167	Male			
	168	Male			
V	170	Female	Dec. 27, 1929	Toy collie	Brought to laboratory Feb. 3, 1930, and placed at once on diet 26
	171	Female			
	172	Female			
Z	185	Male	Jan. 30, 1930	German shepherd	Brought to laboratory Mar. 6, 1930, placed on diet 27. Four other members of this litter were placed on low calcium diet 24 (3)
	186	Male			
	189	Male			
	190	Female			
A1	201	Male	July 9, 1930	Shepherd and collie	Brought to laboratory Aug. 15, 1930, placed on diet 28 on Aug. 23
	202	Female			
	204	Female			
	205	Male			
	206	Female			
	207	Female			
B1	210	Female	Sept. 1, 1930	Shepherd and collie	Brought to laboratory Oct. 10, 1930, placed on diet 28 at once
	212	Male			
	214	Male			

Effect of acid and alkali. Some evidence was found previously (4) that on diets of high Ca:P ratio addition of alkali such as sodium carbonate increased the susceptibility to overdosage phenomena when parathyroid extract was administered. In the present series this effect is not so marked, although as may be seen in table 4, dogs 201, 205, and 202 which received sodium carbonate showed somewhat greater rises in serum calcium than did 204 and 206 which received ammonium chloride. The mean rise per 100 units for the acid dogs was 3.7, for the neutral adult dogs, 5.4, and for

TABLE 3

Effect of parathyroid extract on serum Ca and P of young and adult dogs on normal Ca and P intake

LITTER	DOG	DIET	VITAMIN D	PARATHYROID DOSAGE	DATES (1930–31)	WEIGHT	SERUM ANALYSIS		NUMBER OF ANALYSES	REMARKS
							Ca	P		
				units per day		*kgm.*	*mgm. per 100 cc.*	*mgm. per 100 cc.*		
			2 gm. CLO per	None	Mar. 20–May 2	2.7–6.3	11.3	6.0	3	Bowed legs, normal walk. Active
			kgm. per day	30	May 12–23	7.0–8.2	11.7–13.2	5.9–6.4	2	rickets by radiograph at 74 days
				50	May 24–27					
Z	185	27		50	May 28		16.2	6.2	1	
				50	June 4					
				70	June 5					
				100	June 6	9.3	20.3	5.5	1	Symptoms of overdosage
			2 gm. CLO per	None	Apr. 16–May 11	4.3–6.4	11.7	6.2	3	Bowed legs, normal walk
Z	186	27	kgm. per day	None	May 21–June 5	6.8–9.0	11.7	6.2	3	Normal walk. Better healing than in 185
			None	None	Mar. 20–May 11	2.4–6.5	11.6	5.8	4	Bowed legs
Z	189	27		None	May 21–June 5	7.5–9.8	11.4	4.8	4	Normal walk. "Healing rickets" by radiograph
			None	None	Apr. 16–May 2	4.8–6.7	11.7	6.1	2	Bowed legs
				30	May 12–23	7.4–8.7	11.0	6.5–4.5	2	Normal walk
				50	May 25–27					
Z	190	27		50	May 28		10.9	5.9	1	"Healing rickets"
				50	June 4					
				70	June 5					
				100	June 6	9.7	11.4	4.5	1	No symptoms of overdosage

V	170	26	2 gm. CLO per kgm. per day	None	Feb. 24–Apr. 7	1.3–6.5	11.1	6.6	5	Normal
V	171 172	26	None	None	Feb. 26–Mar. 26	1.3–2.2	11.2	6.4	3	Normal
A1	207	28	2 gm. CLO per kgm. per day	None	Nov. 20–Feb. 27	4.7–8.3	12.2	6.9	5	
				100	Jan. 19	7.7	17.4	5.5	1	
				None	Mar. 13–Apr. 16	9.1–9.1	12.4	6.2	5	Some rachitic deformity of forelegs
				100	Mar. 5	9.1	15.7	5.4	1	
				100	Apr. 1	9.0	19.4	5.2	1	
B1	210	28	None	None	Feb. 24–Mar. 24	8.7–8.8	12.7	5.9	4	
				100	Mar. 16	8.8	18.3	5.8	1	No obvious deformity
				None	Apr. 8–29	8.8–9.3	12.9	5.3	4	
				100	Mar. 30	8.8	19.7	5.6	1	
				100	Apr. 14	9.0	18.9	4.9	1	
B1	212	28	2 gm. CLO per kgm. per day	None	Feb. 20–Mar. 20	8.8–10.7	13.1	8.0	4	
				100	Mar. 12	10.3	16.1	8.0	1	Bowed legs
				None	Apr. 2–May 7	10.3–11.3	12.3	5.7	5	
				100	Mar. 25	10.7	18.5	6.9	1	
				100	Apr. 22	11.2	18.7	6.4	1	
B1	214	28	None	None	Feb. 20–Mar. 29	5.8–6.8	13.3	7.1	5	Less deformity than in 212 and 207
				100	Mar. 13	6.5	18.1	7.0	1	
A1	206	28	2 gm. CLO per kgm. per day	None	Nov. 18–Feb. 10	6.2–9.0	13.0	6.2	6	0.6 gm. NH_4Cl per kgm. per day throughout experiment
				100	Feb. 16	9.3	17.5	6.2	1	
					Mar. 3–Apr. 29	9.9–9.6	12.5	6.3	7	
				100	Mar. 16	9.6	17.4	5.9	1	Femur fractured in cage
				100	Apr. 14	9.6	14.3	4.5	1	Deformed legs, difficulty in standing or walking

TABLE 3—*Concluded*

LITTER	DOG	DIET	VITAMIN D	PARATHYROID DOSAGE	DATES (1930–31)	WEIGHT	SERUM ANALYSIS		NUMBER OF ANALYSES	REMARKS
							Ca	P		
				units per day		*kgm.*	*mgm. per 100 cc.*	*mgm. per 100 cc.*		
			2 gm. CLO per kgm. per day	None	Nov. 20–Feb. 24	6.8–10.8	12.0	6.8	6	0.6 gm. NH_4Cl per kgm. per day throughout experiment
				100	Feb. 9	10.4	18.0	5.3	1	
A1	204	28		None	Mar. 3–Apr. 21	10.9	13.1	7.4	6	
				100	Mar. 9	10.9	15.9	6.6	1	Extremely deformed long bones, lame
				100	Apr. 6	10.6	15.2	3.6	1	
			None	None	Nov. 20–Feb. 24	6.2–10.8	11.1	6.9	4	0.8 gm. Na_2CO_3 per kgm. per day given throughout experiment
				100	Feb. 9	10.3	18.9	6.8	1	
A1	201	28		None	Mar. 3–Apr. 21	11.0–12.8	12.4	6.2	6	
				100	Mar. 9	11.7	16.2	6.4	1	
				100	Apr. 6	12.3	18.4	6.3	1	No rachitic deformity
			2 gm. per kgm. per day	None	Dec. 15–Mar. 10	8.4–11.9	12.8	6.8	6	Also given Na_2CO_3 as was 201. No rachitic deformity, although some weakness in hind legs
				100	Mar. 2	11.0	20.6	7.7	1	
A1	205	28		None	Mar. 17–May 12	12.0–11.9	12.4	6.2	7	
				100	Mar. 30	11.9	17.6	4.7	1	
				100	Apr. 27	11.9	14.8	7.6	1	
			2 gm. per kgm. per day	None	Dec. 19–Mar. 10	7.7–8.9	11.5	7.2	5	Also given Na_2CO_3 as was 201
				100	Feb. 16	8.9	20.0	6.8	1	
A1	202	28		None	Mar. 24–Apr. 29	9.4–9.6	11.8	5.6	5	No rachitic deformity
				100	Mar. 16	9.2	18.1	6.2	1	
				100	Apr. 14	9.3	17.8	3.4	1	

TABLE 4

Rise in serum calcium due to parathyroid extract as influenced by age and reaction of diet

DOG	VITAMIN D AND REACTION OF DIET	PARATHYROID DOSAGE	AGE	RISE IN SERUM CALCIUM
		units per day	*days*	*mgm. per 100 cc.*
185	+, neutral	30	102	1.9
		50	118	4.9
		100	127	9.0
190	−, neutral	30	102	−0.7
		50	118	−0.8
		100	127	−0.3
207	+, neutral	100	194	5.2
		100	239	3.3
		100	266	7.0
210	−, neutral	100	195	5.6
		100	209	6.8
		100	224	6.0
212	+, neutral	100	191	3.0
		100	204	6.2
		100	232	6.4
214	−, neutral	100	192	4.8
204	+, acid	100	215	6.0
		100	243	2.8
		100	271	2.1
206	+, acid	100	222	4.5
		100	250	4.9
		100	279	1.8
201	−, alkaline	100	215	7.8
		100	243	3.8
		100	271	6.0
205	+, alkaline	100	236	7.8
		100	264	5.2
		100	292	2.4
202	+, alkaline	100	222	8.5
		100	250	6.3
		100	279	6.0

the alkaline dogs, 5.9. The mean rise of all adult dogs with cod liver oil was 5.0 and without cod liver oil was 5.8. The difference is probably significant only in the case of the acid dogs, which showed definitely lower responses than did the neutral and alkaline.

The diet used in standardizing the extract. An excellent check was thus obtained upon the so-called standardized value (5) of the parathyroid extract when young adult dogs were used with diets of normal Ca:P ratio of neutral reaction either with or without vitamin D. The same diet fed to young pups, 185 and 190, apparently produced an exaggerated or a negative response in accordance with the presence or absence of vitamin D. The use of adult dogs, normal Ca:P ratio of diet and approximately neutral reaction appears, therefore, to be desirable for such standardization.

Of the nineteen determinations made on seven dogs under these conditions, the mean was 5.5 mgm. increase in serum calcium per 100 units injected with probable error of ± 0.25 and standard deviation of 1.6. It is possible that the varying amount of hypercalcemia produced with acid or alkaline diets of high and normal Ca:P ratio is due to purely physicochemical relations of calcium phosphate. The possible course of events in hyperparathyroidism has been pictured (6) as follows: 1, increased phosphate production and therefore increased plasma phosphate concentration whether due to more complete absorption from the intestine or release from the tissues; 2, increased phosphate excretion followed by: 3, a decrease in plasma phosphate, this in turn allowing 4, a rise in serum calcium, which may be followed or accompanied by 5, increased calcium excretion or deposition in tissues. If each of these steps following the first be considered dependent upon solubility relations of calcium phosphate it is obvious that variations in the acidity of the circulating fluid should affect the sequence of events inasmuch as they affect that solubility. An increase in blood acidity due to ingestion of ammonium chloride might interfere with the rise in serum calcium by increasing the rate of deposition or excretion of calcium phosphate more markedly than it does the calcium dissolving power of the serum, whereas sodium carbonate might produce the opposite effect by a corresponding decrease in such deposition or excretion.

Attention is drawn to the greatly increased degree of deformity seen in the young dogs fed ammonium chloride along with the normal basal diet and cod liver oil as compared with the development of the neutral and sodium carbonate-fed animals. Thus, as shown in figure 1, dogs 204 and 206, acid-fed, had extremely bowed long bones while 202 and 201, alkali-fed, were nearly normal. This is similar to the conditions observed in an earlier study (7), in which dogs on normal diet were subjected to the effects of acid and alkali ingestion both with and without cod liver oil. The large rapidly growing breeds of dogs, when given acid under these circumstances develop extremely severe bone lesions with permanent de-

formities, whereas the neutral and alkali-fed dogs have much less serious lesions.

Metabolic balances on normal Ca:P ratios. The metabolic balances carried out on three of these dogs of litter Z as shown in table 5 indicated but little difference in calcium retention due to vitamin D, since dogs 189 and 190 without cod liver oil retained in periods 1 and 2 almost the same amounts absolutely and relatively to intake as did 185 with cod liver oil. During periods 3 and 4 also when parathyroid extract was given to 185 and 190 the retention of calcium seemed little affected in either animal. The phosphorus retention likewise in periods 1 and 2 was much the same in the three dogs but in periods 3 and 4 that of dog 185 was somewhat decreased, although that of dog 190 was either not affected or slightly improved. None of the changes are striking, however. It appears that on an adequate diet of this type and of this slightly low Ca:P ratio, either with or with-

204 202 206 201

Fig. 1. Effect of addition of acid and alkali to diet of young dogs fed a normal diet. Note extreme deformity of acid-fed dogs 204 and 206. Dogs 202 and 201 were given alkali.

out vitamin D, 70 to 92 per cent of the calcium and 24 to 38 per cent of the phosphorus is retained by the growing dog. These figures are similar to those obtained by Shohl and Bennett (8).

Ca:P ratio of the balances. The striking contrast of this apparent lack of effect of vitamin D on balances with the normal diet with that shown in the low calcium-high phosphorus diet (3) illustrates again the importance of the Ca:P ratio as compared with that of the absolute quantity of these elements in the diet. It will be observed that the Ca:P ratio of the balances on the normal diet (table 5) are within the normal range for bone growth, 1.5 to 2.0, except in period 4 of dog 185 when the large dosage of parathyroid extract exerted a reducing effect upon the phosphorus retention. On low calcium intake, this ratio is for the most part less than 1, except again in the high parathyroid period of dog 187, receiving cod liver oil. The retention may be said to follow the ratio of the diet whenever the latter is abnormal and to assume the bone-building ratio of normal calcium

phosphate whenever the intake of these elements approaches the proportion of 1 to 1. This supports the assumption that reserve deposits of both elements are constantly and almost instantaneously made somewhere in the tissues, possibly in the bony trabeculae as was shown by Bauer, Aub and Albright (9). These investigators were not able to obtain hypercalcemia in cats or kittens by the use of parathyroid extract, a fact explicable probably in terms of diet and vitamin D. The diet is not described in

TABLE 5

Ca and P exchange on normal Ca and P intake as affected by parathyroid extract

Milligrams per kilogram per day

DOG	PERIOD	DATES (1930)	WEIGHT	PARATHYROID EXTRACT	INTAKE		OUTPUT				BALANCES		PER CENT OF INTAKE RETAINED	
							Ca		P					
					Ca	P	Urine	Feces	Urine	Feces	Ca	P	Ca	P
			kgm.	*units per day*										
185*	1	Apr. 16–23	5.1–5.5		91	132	1	6	81	1	84	50	92	38
	2	Apr. 26–May 1	5.5–6.2		116	163	2	7	82	18	107	63	92	38
	3	May 8–14	7.0–7.5	15	86	124	1	5	67	12	80	45	93	36
	4	May 15–23	7.0–8.2	30	93	147	2	9	109	20	82	16	88	10
189	1	Apr. 16–23	4.6–5.7		132	186	1	39	92	29	92	55	70	29
	2	Apr. 24–May 1	5.7–6.5		111	180	3	9	99	18	99	63	89	35
	3	May 8–14	6.5–7.5		138	193	3	16	101	22	119	70	86	36
	4	May 15–23	7.5–9.1		114	175	2	12	98	27	100	50	87	28
190	1	Apr. 16–23	4.8–5.3		134	192	4	33	103	38	97	51	72	26
	2	Apr. 24–May 1	5.3–6.3		134	187	6	17	100	29	111	58	83	31
	3	May 8–14	6.7–7.4	15	137	193	3	18	92	26	116	75	84	38
	4	May 15–23	7.4–9.1	30	117	182	2	23	96	41	92	44	79	24

* 185 received 2 grams cod liver oil per kgm. per day.

any case, but if made up largely of meat would provide acid reaction, low calcium and vitamin D-free conditions, all of which tend to decrease the response to parathyroid. The rabbits in which hypercalcemia was observed were given a high calcium diet which may have contained enough vegetables to furnish an alkaline reaction with corresponding increase in hypercalcemia. The increase in trabeculae shown by the young rats to which they administered parathormone is paralleled by the positive line tests obtained in certain experiments in this laboratory with rats on rachitogenic diets (high calcium-low phosphorus) without vitamin D (2).

Paired feeding test with litter U. Six young well-bred airedales of litter U were placed on experimental diet at 82 days of age. Two females, nos. 163 and 164, were given diet 22, high calcium-low phosphorus, dog 163 being given in addition tested viosterol (100 D) in the amount of 10 and

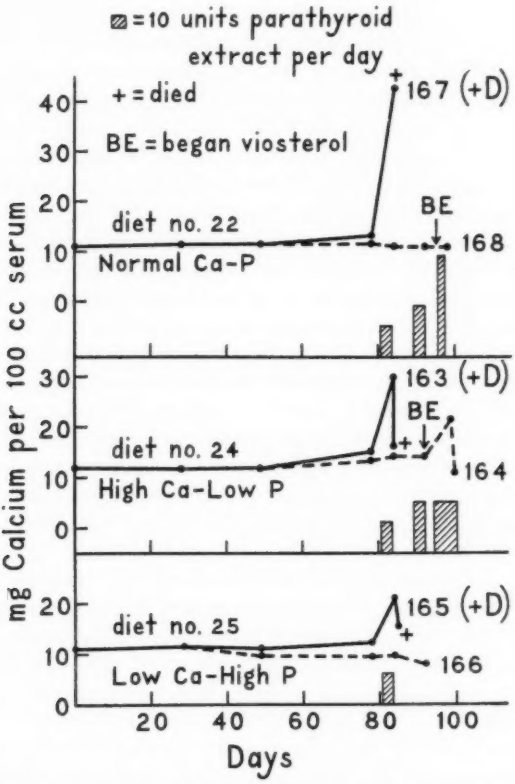

Fig. 2. Effect of varying Ca:P ratio of diet and of viosterol upon the serum calcium of 3 pairs of young dogs of the same litter following parathyroid extract injection. All except 168 weighed 2 to 3 kgm. at 3 months of age when the serum analyses were begun and 4 to 5 kgm. 81 days later when the first parathyroid extract injections were made. Dog 168 weighed 3.2 and 8.0 kgm. on the days mentioned. All showed "moderate to severe rickets" by x-ray.

then 20 mgm. per kgm. per day. On the smaller dosage after 33 days severe rachitic depression developed which gradually cleared up when the dosage of viosterol was doubled. Dog 164 was said to show "severe active rickets" by x-ray diagnosis. On the 81st, 82nd and 83rd days on diet,

April 27 to 29, each of the animals was given 30 units of parathyroid extract. Dog 163 at once showed symptoms of overdosage, hypotonia, loss of appetite and extremely high serum calcium. On the third day it died of overdosage. The usual gastric and intestinal hemorrhages were found. Dog 164 on the other hand withstood the treatment showing only a slight rise in serum calcium (fig. 2). Fifty units of the extract were injected each day, May 5 to 7, again without appreciable response. On May 11 to 14, 20 mgm. of viosterol per kgm. and 50 units of parathyroid extract were given simultaneously with resulting hypercalcemia and death from overdosage.

This experiment appears to show that vitamin D exerts its effect at once in allowing the development of high serum calcium following parathormone administration when a sufficiently high Ca:P ratio is present in the diet. Even if it be assumed that vitamin D acts through the parathyroids this experiment indicates also that no serious injury to the parathyroid apparatus had been produced by the long continued lack of vitamin D.

Dogs 165 and 166, females, also members of litter U, were given diet no. 24, low calcium-high phosphorus, 165 receiving viosterol in addition as did 163. Dog 166 developed lower serum calcium than did any of the others although 164 showed lower phosphate. Again parathyroid was given as for the first pair with immediate fatal overdosage in the case of 165, but with no effect upon 166. Unfortunately at this time 166 began to show serious distress from hernia so that it was deemed unwise to attempt the administration of viosterol and parathyroid simultaneously. It is interesting that the viosterol and parathyroid produced immediate overdosage in dog 165 on the low calcium diet which with cod liver oil gave only a moderate hypercalcemia in other animals, apparently indicating the accumulation of a vastly superior calcium reserve or the presence of some other predisposing factor under the influence of the viosterol. Shelling and Asher (10) found that optimal calcium-high phosphorus diet was most injurious to rats when overdosage with viosterol occurred.

Dogs 167 and 168, males, were placed on normal diet no. 25, of Ca:P ratio 1.2, 167 being given viosterol. When 30 units of parathyroid extract were given daily on April 27 to 29, dog 167 succumbed on the third day with the extraordinarily high serum calcium of 42.6 mgm. per cent. Dog 168 on the other hand showed no effect then nor even when 50 units were given daily on May 5 to 7. On May 12 and 13, 50 units were given simultaneously with 20 mgm. viosterol (100 D) per kgm. daily. No rise in either serum calcium or phosphate resulted although there were some symptoms of overdosage. On May 14 the dog was sacrificed and lung abscesses discovered but no intestinal hemorrhages. This result when compared with that obtained with dog 164 on high calcium-low phosphorus diet might be thought to point to lower calcium reserve in the animal on normal cal-

cium intake or to the overweaning importance of the high Ca:P ratio of the diet in establishing susceptibility to the combined action of vitamin D and parathyroid. On nearly the same Ca:P intake dog 152 (1) had shown no hypercalcemia on parathyroid treatment when cod liver oil was added to the diet after a long period without vitamin D.

SUMMARY. 1. A diet of normal Ca:P ratio both with and without vitamin D produced normal serum calcium and phosphate in young dogs.

2. Injection of parathyroid extract brought about continued hypercalcemia in young dogs on the normal diet with vitamin D, but little response without the vitamin. However, older animals responded almost equally with and without the vitamin.

3. When ammonium chloride was added to the diet, the hypercalcemia produced by a given dose of the parathyroid extract was reduced somewhat, while on addition of sodium carbonate some increase was noted.

4. Calcium and phosphorus retention were nearly the same on the normal diet with and without vitamin D. In the presence of the vitamin, parathyroid treatment in the dosage used produced a lowered phosphorus retention, but no effect when vitamin D was not present.

5. The Ca:P ratio of the balances tended to approach the bone ratio of 1.5 to 2.

6. Three pairs of young dogs of the same litter fed these three diets, high calcium-low phosphorus, low calcium-high phosphorus and normal, in each case one with and one without viosterol, responded to parathyroid treatment in precisely the fashion to be expected if the response is dependent upon the presence of a calcium reserve.

7. When viosterol and parathyroid extract were given simultaneously to dogs previously non-responsive because without vitamin D, immediate hypercalcemia resulted in one on high calcium diet but little change in that on normal diet.

CONCLUSIONS

1. Vitamin D-free young dogs on all diets and all dogs on low calcium diets respond with little hypercalcemia to parathyroid extract injection because they lack, or are easily stripped of, mobilizable calcium reserve. Adult dogs on diets of normal Ca:P ratio may accumulate sufficient reserves to respond to parathyroid treatment normally regardless of vitamin D intake.

2. Vitamin D does not act by stimulation of the parathyroid glands but by increasing the calcium supply either from the gut or bone trabeculae from which are drawn the materials for ossification. Such increase may be almost instantaneous if the diet contains a large calcium content and particularly if the Ca:P ratio is high.

3. Essential bony tissues are not necessarily sacrificed by parathyroid

treatment as is shown by the abnormally high calcium to phosphorus ratio of the decreased retentions resulting from such treatment.

4. The source of the excess serum calcium in the hypercalcemia following parathyroid extract injection in moderate dosage is the calcium of the diet or of a rapidly shifting calcium reserve rather than of the essential bony structures.

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STUDIES ON MAGNESIUM DEFICIENCY IN ANIMALS

IV. Reaction to Galvanic Stimuli Following Magnesium Deprivation

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The restriction of young rats to a ration containing only 1.8 parts per million of magnesium, but adequate amounts of other ingredients, brings on a striking series of symptoms with a violently fatal outcome (1). The sequence of events is: vasodilatation, hyperexcitability, tonic-clonic convulsions, and death. By the fourth day on the deficient diet the skin surface of the animals is tinged with redness due to vasodilatation; then the coloration slowly intensifies only to gradually fade and disappear by the 12th day; thereupon sets in hyperirritability of the nervous system which is indicated by the facility with which noises throw the animals into tonic-clonic convulsions; finally, by the 21st day such seizures usually bring a fatal termination. Essentially the same symptomatology, with an understandable nuance in the intensity of some features, has been evidenced in another species (2).

These effects upon the nervous system, designated as a local manifestation, were recognized as a form of tetany, which by definition is a symptom-complex characterized by hyperirritability, spasm, and tonic-clonic convulsions. In its latent form, with absence of frank symptoms, tetany is detected by the response to weak galvanic stimuli, a reaction disclosing the increased sensitivity of the nervous system. In a previous communication we advanced three reasons for not testing the electrical reactions in rats on a magnesium-low diet (1). We stated:

> The animals are so hyperexcitable that no such sensitive method is needed. When, as we have repeatedly observed, noises that leave the control animals undisturbed throw the experimental animals into convulsions, it is evident that stimuli other than electricity serve to indicate the extreme irritability of the nerves. Secondly, the irritable condition in our rats always leads to convulsions; i.e., symptoms so readily recognizable that electrical excitation is not necessary. Finally, the growing distrust among investigators in the dependability of the electrical reactions seriously impairs their value.

Our views in this matter have since suffered no change. Nevertheless, because some investigators, at least, give much credence to the electrical

reactions, and because a second line of evidence, even if seemingly superfluous, should serve to substantiate definitely the diagnosis of tetany, we have determined the neuromuscular response of young rats to galvanic stimuli during the period of magnesium deprivation.

TECHNIQUE. Fifteen rats, 27 days old and between 30 to 45 grams in weight, were divided into two groups, five in one group and ten in the other, with the sexes comparably distributed. After two preliminary determinations of their reactivity to galvanic stimuli on successive days, during which time all were given the stock diet, the two groups were separated. The lot of ten, restricted to the magnesium-low diet (diet 10), served as the experimental group, and the lot of five, given the magnesium-low diet plus added $MgSO_4 \cdot 5H_2O$ (diet 11), served as the control group. The composition of the rations has already been described (1). Following their restriction to the respective rations, both groups were subjected to the electrical test twice weekly until the end of the survival period in the case of the experimental animals and for a slightly longer time in the case of the controls. The technique of conducting the test was patterned after the plan of Shohl and Bing (3).

RESULTS. In the normal animals the strength of current required to produce a contraction was different for each kind of shock; accordingly an ascending series may be formed in the order C.C.C. < A.C.C. < A.O.C. < C.O.C. Never was the intensity of current for an A.O.C. less than that for an A.C.C. (table 1). That the values for the electrical reactions in rats undergo change with age, an already accepted relationship in humans, is demonstrated by the data derived from the control animals. Although the range of age in these rats extended only from the 27th to the 83rd day, a period of 55 days, it is seen that with increasing age a weaker current sufficed to elicit a contraction, and the diminution was reflected *pari passu* in each kind. When the average of the values for the four kinds of current, taken from the five animals at each particular age, is plotted, the gradual lowering of the threshold of nervous sensitivity with progressing age becomes even more readily evident (chart 1). The general course of the curve, plotted from such mean figures, is a gentle downward slope, to which the values for both sexes conform.

So similar in character were the values for the electrical reactions obtained from the ten animals restricted to the magnesium-deficient diet, that for purposes of condensation the results of five, selected only insofar as to represent a comparable sex distribution to that in the controls, have been prepared in tabular and graphic form. During the first 11 days that these animals were restricted to the deficient ration, the figures for the strength of each kind of current to which they responded with muscular contraction were not unlike those recorded for control animals of the same age (table 2). Thereafter the values fell rapidly and approached a level

The strength of current, measured in milliamperes, necessary to induce a neuromuscular response in rats over a 55-day period during which they were given the control diet (magnesium-deficient ration plus added $MgSO_4 \cdot 5H_2O$—diet 11)

	♂ YELLOW HEAD	♀ ALBINO	♀ BLACK HEAD	♀ BLACK	♂ BLACK

The strength of current, measured in milliamperes, necessary to induce a neuromuscular response in rats over a 55-day period during which they were given the control diet (magnesium-deficient ration plus added $MgSO_4 \cdot 5H_2O$,—diet 11)

DAYS ON DIET	AGE	♂ YELLOW HEAD				♀ ALBINO				♀ BLACK HEAD				♀ BLACK				♂ BLACK			
		C.C.C.	A.C.C.	A.O.C.	C.O.C. or C.C.Te.	C.C.C.	A.C.C.	A.O.C.	C.O.C. or C.C.Te.	C.C.C.	A.C.C.	A.O.C.	C.O.C. or C.C.Te.	C.C.C.	A.C.C.	A.C.C.	C.O.C. or C.C.Te.	C.C.C.	A.C.C.	A.O.C.	C.O.C. or C.C.Te.
days	*days*	*ma.*	*ma.*	*ma.*	*ma.*	*ma.*	*ma.*	*ma.*	*ma.*	*ma.*	*ma.*	*ma.*	*ma.*	*ma.*	*ma.*	*ma.*	*ma.*	*ma.*	*ma.*	*ma.*	*ma.*
Prelim.	27	0.38	0.54	0.76	1.34	0.18	0.64	0.80	1.36	0.20	0.46	0.88	1.20	0.28	0.40	0.98	1.68	0.28	0.42	0.96	1.30
Prelim.	28	0.34	0.62	1.16	1.32	0.26	0.48	0.96	1.12	0.26	0.52	0.76	1.20	0.32	0.52	1.08	1.40	0.26	0.42	0.88	1.30
4	32	0.32	0.60	1.00	1.28	0.28	0.72	1.20	1.70	0.36	0.56	1.20	1.40	0.36	0.68	1.20	1.60	0.30	0.65	1.00	1.60
7	35	0.24	0.46	0.90	1.20	0.20	0.55	0.85	1.20	0.25	0.45	0.90	1.30	0.28	0.62	1.00	1.30	0.24	0.46	0.80	1.10
11	39	0.26	0.40	0.80	1.20	0.22	0.44	0.80	1.20	0.32	0.56	0.90	1.30	0.28	0.45	0.90	1.20	0.30	0.58	0.70	1.20
14	42	0.26	0.44	0.90	1.20	0.28	0.52	0.80	1.30	0.28	0.44	0.70	1.20	0.26	0.50	0.90	1.10	0.32	0.56	0.90	1.30
18	46	0.25	0.38	0.90	1.20	0.28	0.44	0.80	1.10	0.22	0.32	0.60	1.40	0.24	0.40	0.90	1.10	0.28	0.38	0.90	1.10
21	49	0.22	0.40	0.76	1.10	0.24	0.38	0.80	1.28	0.18	0.28	0.60	1.40	0.20	0.38	0.90	1.20	0.24	0.38	0.70	1.10
25	53	0.20	0.38	0.90	1.20	0.26	0.40	1.00	1.40	0.18	0.30	0.90	1.40	0.26	0.40	0.70	1.20	0.25	0.38	0.90	1.20
28	56	0.26	0.44	0.80	1.00	0.18	0.42	0.88	1.20	0.22	0.36	0.76	1.30	0.24	0.48	0.80	1.10	0.20	0.36	0.96	1.40
32	60	0.22	0.36	0.90	1.10	0.20	0.36	0.80	1.20	0.22	0.36	0.70	1.10	0.24	0.40	0.80	1.20	0.28	0.42	1.00	1.20
42	70	0.20	0.37	0.78	1.00	0.20	0.32	0.80	1.30	0.16	0.28	0.70	0.80	0.20	0.36	0.70	1.20	0.18	0.34	0.90	1.30
48	76	0.20	0.40	0.80	1.30	0.26	0.34	0.80	1.20	0.22	0.28	0.70	1.20	0.26	0.36	0.90	1.20	0.25	0.34	1.00	1.30
55	83	0.18	0.28	0.90	1.10	0.18	0.28	0.68	1.00	0.22	0.28	0.50	1.00	0.24	0.38	0.80	1.30	0.27	0.36	0.90	1.00

TABLE 2

The strength of current, measured in milliamperes, necessary to induce a neuromuscular response in rats during the period of their survival on a magnesium-deficient ration (diet 10)

DAYS ON DIET	AGE	♀ BLACK-A				♀ YELLOW HEAD-B				♂ YELLOW HEAD-C				♂ YELLOW-D				♀ YELLOW-E			
		C.C.C.	A.C.C.	A.O.C.	C.O.C. or C.C.Te.	C.C.C.	A.C.C.	A.O.C.	C.O.C. or C.C.Te.	C.C.C.	A.C.C.	A.O.C.	C.O.C. or C.C.Te.	C.C.C.	A.C.C.	A.O.C.	C.O.C. or C.C.Te.	C.C.C.	A.C.C.	A.O.C.	C.O.C. or C.C.Te.
days	*days*	*ma.*	*ma.*	*ma.*	*ma.*	*ma.*	*ma.*	*ma.*	*ma.*	*ma.*	*ma.*	*ma.*	*ma.*	*ma.*	*ma.*	*ma.*	*ma.*	*ma.*	*ma.*	*ma.*	*ma.*
Prelim.	27	0.22	0.42	0.90	1.40	0.26	0.36	1.00	1.30	0.30	0.38	0.90	1.20	0.36	0.58	0.96	1.40	0.24	0.44	0.90	1.50
Prelim.	28	0.26	0.50	0.96	1.40	0.32	0.48	1.00	1.30	0.26	0.50	1.10	1.30	0.32	0.54	1.00	1.32	0.26	0.48	1.00	1.30
4	32	0.22	0.50	0.90	1.40	0.22	0.38	0.70	1.30	0.32	0.64	0.60	1.20	0.28	0.58	1.20	1.50	0.32	0.60	1.00	1.50
7	35	0.28	0.50	1.00	1.20	0.28	0.55	0.80	1.20	0.26	0.60	1.00	1.30	0.18	0.50	1.10	1.40	0.25	0.55	1.00	1.30
11	39	0.28	0.56	0.90	1.20	0.28	0.48	0.90	1.20	0.20	0.40	0.90	1.04	0.34	0.56	0.90	1.20	0.16	0.30	0.74	1.00
14	42	0.16	0.42	0.70	1.00	0.18	0.28	0.60	0.70	0.18	0.38	0.84	0.74	0.18	0.40	0.70	1.00	0.16	0.18	0.40	0.50
18	46	0.16	0.24	0.60	0.70	0.18	0.24	0.60	0.50	0.26	0.42	0.90	0.96	0.18	0.38	0.54	0.40				
21	49	0.14	0.18	0.64	0.50	0.12	0.16	0.50	0.40	0.16	0.20	0.48	0.44								
25	53	0.18	0.26	0.60	0.60	0.12	0.20	0.68	0.70												
28	56	0.18	0.30	0.76	0.90	0.12	0.15	0.48	0.44												
32	60	0.16	0.30	0.90	0.70	0.10	0.14	0.40	0.40												
42	70	0.14	0.30	0.76	0.60	0.10	0.20	0.60	0.50												

much below not only the previous figures for the same animals but also the figures for the controls of similar age. For example, the control animals at 49 days of age had, on the average, values of 0.24 ma., 0.38 ma., 0.80 ma., and 1.20 ma., for the reactions C.C.C., A.C.C., A.O.C., and C.O.C. respectively; whereas animal B at the same age, having been 21 days on the magnesium-deficient diet, showed values of 0.12 ma., 0.16 ma., 0.50 ma., and 0.40 ma. respectively. The contrast between these diminished values in the experimental animal and its own high initial values at

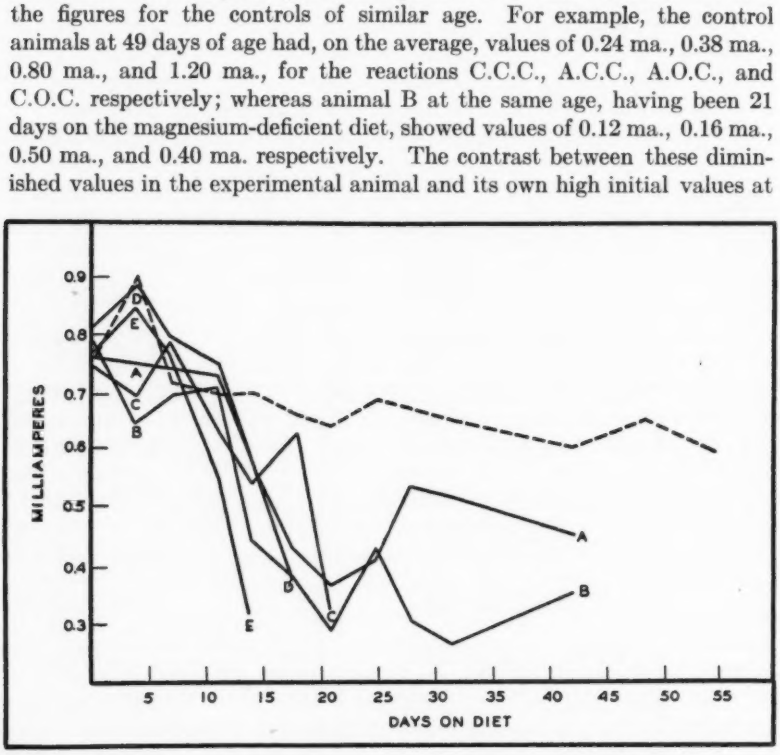

Chart 1. The strength of current, representing the average of C. C., A. C., A. O., and C. O. values, necessary to induce a neuromuscular response at various intervals in rats restricted to a magnesium-deficient ration as contrasted with that in rats given the control diet containing adequate magnesium. The averages for each rat restricted to the magnesium-deficient ration are shown by individual curves with solid lines, the letter at the end of each curve identifying the animal. The averages for the five control rats are shown in the single curve with the broken line.

27 days of age, prior to deprivation of magnesium, is even more conspicuous, so that the diminution in strength of current necessary for contraction, as a result of magnesium deficiency, is unmistakable. Although the figures for all the types of current generally fell at the same time, in certain reactions the decline was sharper than in others; for in practically every case where low values prevailed C.O.C. was less than A.O.C., a condition which might be termed closing reversal. Convulsions, usually fatal in their outcome, set in when such a low level. with or without

closing reversal, was reached. If the animals survived this seizure, for a short time thereafter the values climbed slowly but never attained a normal plane; then they soon dropped again to the level productive of a fatal attack.

These changes in the electrical reactions, appearing in the course of magnesium deficiency, are likewise best appreciated by plotting for each animal for each particular day on the diet the average strength of the four kinds of current requisite for a muscular response, since the curve for each animal on the deficient diet may be contrasted with that for the control animals, thereby excluding age as a factor (chart 1). By this method it becomes apparent that the average figure for each experimental animal during the first eleven days on the deficient ration is the same as for the controls, and that both are likewise in excess of 0.6 ma. On the 14th day of restriction to the magnesium-deficient diet, the animals showed a precipitous drop in the intensity of current necessary for nerve stimulation, with values such as 0.45 to 0.55 ma. placing them in the hyperirritability zone. By the 21st day the low point in the curve was reached with such figures as 0.29 to 0.36 ma., although one animal, apparently more susceptible than its mates to magnesium deprivation, reached this minimum level on the 18th day. In sharp contrast to these low average figures for the strength of current necessary to produce a contraction on the 21st day, stands that for the control animals of the same age; namely, 0.65 ma. Three animals, deprived of magnesium, succumbed to fatal convulsions on or before the 21st day, coincident with the drop to the low point of their curves (chart 1). Due to the violent nature of the attacks, no reactions were taken during the stage of frank convulsions. Two animals, A and B, with minimal average values on the 21st day, had convulsions which they survived; thereafter their curves rose slightly for a brief period, only to decline again later to an extremely low level with fatal seizures.

Discussion. The galvanic reactions have been investigated most frequently in humans, particularly infants and children, for whom normal values at various ages have been established (4). Although the figures for humans have scant application as a basis for predicting absolute values in the rat, they are useful for purposes of comparison with values from the latter species on such points in the relationship and behavior of the electrical reactions as: their usual order of arrangement according to strength of current, with instances of changes; their trend with increasing age; and their degree of reliability. It has been noted in humans, for instance, that the four kinds of current, arranged in the order of intensity necessary for muscular contraction, formed the ascending series C.C.C. < A.C.C. < A.O.C. < C.O.C. Yet with gradually increasing frequency, age in its course brought in inversion of the usual anodal relationship so that less current served for an A.O.C. than for an A.C.C. As a matter of fact, the

high electrical values of early infancy slowly decline to reach the low level of adulthood. With due consideration to all of these items, it should be not forgotten that a wide normal variation must be taken into account.

In only one instance have the electrical reactions been applied to normal rats (3). There the requisite strength of each kind of current capable of inducing muscular response was much less than for infants but the arrangement of the different kinds in a series, according to effective strength, was the same. Anodal reversal, however, was seldom seen; indeed, just once. From determinations on different animals of various ages, a method identical with that practiced upon humans, the same lowering of the nervous threshold with progressing age was met. When our data in turn are compared with that just enumerated for the normal rat, we find the same order of reactions, the same absence of anodal reversal, the same lessened stimulus with increasing age, insofar as it was ascertained during the short span of 8 weeks. Our absolute figures for currents setting up contractions in the normal animals are in satisfactory agreement with the previously recorded results for this species, when allowance is made for differences in diet and personal factors in the two cases.

Just as normal values and relationship for electrical reactions have been derived almost exclusively from humans, so in tetany by far the greatest part of the aggregate of data, which has been adequately reviewed, was drawn from a similar source (4, 5, 6). As an outgrowth of a succession of studies, several characteristic changes in the reactions have been advanced as pathognomonic of tetany and recommended as diagnostic aids. At the present time, however, only two may claim our attention: anodal reversal and an exaggerated C.O.C. Although anodal hyperexcitability first came into consideration in tetany because it preceded, accompanied, and outlasted cathodal hyperexcitability, it received emphasis later because of its peculiar nature wherein A.O.C. occurred with a weaker current than for A.C.C. (4). But this anodal reversal now holds only secondary significance since the lowered threshold for C.O.C. has been pronounced the most serviceable reaction (5).

In contrast to the abundant records of electrical reactions in human tetany, the figures for hyperirritability in the rat are few. After perfecting the technique applicable to the latter species, Shohl and Bing made determinations in several disorders of dietary origin. By sudden lowering of the high Ca:P ratio in a rachitogenic diet, they encountered in the rat a lowered threshold of neuromuscular irritability indicative of tetany (7). Not only were the absolute values in this condition, as was to be expected, much lower in magnitude than those prevailing in human tetany, but also relative changes appeared that were unlike the usual course of events in the latter syndrome. In the general downward trend of the values with the onset of hyperexcitability, the decrease in the anodal currents was

never such as to occasion anodal reversal, but so disproportionately did the C.O.C. figures fall that they became less than the A.O.C. values.

Despite the fact that Shohl and Bing, using abrupt amendment of a Ca:P imbalance, induced hyperirritability by an entirely different mechanism than that resulting from magnesium deficiency, quantitative results and relationships in the two cases are not essentially different. In both, anodal reversal is absent and C.O.C. occurs with a weaker current than is required for A.O.C. The low values for the latent stage of calcium tetany find a counterpart in the figures from our animals just prior to an attack; the minimal values obtained while the former animals were in convulsions cannot be matched by figures for a similar occasion in magnesium tetany, since the violent nature of the attacks precluded any electrical determinations. In the two conditions common points in the electrical reactions support the forceful proposition that magnesium deficiency, as truly as calcium disorder, leads to tetany.

While such a comparison, dealing with quantitative data, is useful in establishing the hyperirritability of magnesium deficiency as tetany, it may be misleading unless two factors concerning the electrical reactions in general are borne in mind. First, the measure of current eliciting a muscular response bears no relation to the severity of symptoms in tetany, according to Hess (5). It would seem futile, therefore, to wield figures in an effort to ascertain the relative severity of calcium and magnesium tetany. Secondly, the measure of current denoting the presence of hyperirritability tells nothing concerning the mechanism at work; it establishes the existence of tetany without elucidating its etiology. Tetany evolves through several unrelated agencies. For insight into the cause and development of hyperexcitability in a particular instance, there must be recourse to means other than the electrical reactions. In the type of tetany resulting from abrupt change of calcium imbalance in the ration, it is accepted that a temporary lowering of the serum calcium is responsible for the hyperirritability (8); in the type appearing in magnesium deficiency, it is known from blood studies that calcium plays no part and that diminished concentration of magnesium in the serum is the sole factor (9). With due consideration to the quantitative aspects of the absolute and relative changes in the electrical reactions, the principal point is that all the reactions are accomplished with less current at a definite period in magnesium deprivation; from this fact, accordingly, there is permissible only the inference that the hyperirritability characterizing magnesium deficiency is really tetany.

That the mensurable method involving electric stimuli bears out in a general way our previous impression, gained solely from the effects of auditory stimuli on animals deprived of magnesium, is the matter of prime interest at the moment, but the extent to which the observations from the

two approaches coincide in their details likewise warrants mention. From the description of symptomatology in magnesium tetany, published previously, it may be recalled that hyperexcitability in the animals becomes progressively more pronounced from the 12th to the 18th day; and that "although convulsive attacks may appear as early as the 11th day, the more usual time is the 18th day, and by the 23rd day practically all animals have had the first seizure." Using the sound from a blast of air as an excitant in our earlier work, we were able to induce convulsions in the animals never before their 11th day on the magnesium-deficient diet, only rarely then, and most frequently on the 18th day. It is apparent that the appearance of hyperexcitability is detected at this same time by the use of electric shock, for weaker currents then suffice to evoke a neuromuscular response. For instance, the curves depicting the strength of stimulus necessary for a contraction fall on the 14th day and reach minimal points on the 21st day. In all, whether in its general nature or in its particulars, the utilization of the electrical reaction furnishes a cogent substantiation of qualitative observation by quantitative measurement.

SUMMARY

By restriction to a magnesium-deficient ration, the rat's threshold of sensitivity to electrical stimuli is lowered, since very weak currents elicit a neuromuscular response. Such hyperirritability, detectable in mensurable units by the electrical method, satisfies the criterion for the diagnosis of tetany as a manifestation of magnesium deficiency. When determined by electrical reactions, the time at which hyperirritability appears corresponds to that previously ascertained by use of auditory stimuli.

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THE EFFECT OF THE CONTINUOUS INTRAVENOUS INJECTION OF RINGER'S SOLUTION ON THE BLOOD CHEMISTRY OF THYRO-PARATHYROIDECTOMIZED DOGS[1]

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In 1921, Luckhardt and Rosenbloom and one of us (L. R. D. 1922) found that thyro-parathyroidectomized dogs could be kept alive indefinitely and free from tetany by treatments that did not involve the administration of any form of parathyroid substance. Since at that time it was currently believed that calcium therapy would afford only a temporary relief of parathyroid tetany and not prolong life (Marine, 1914; Voegtlin, 1917, and Biedl, 1922) the authors believed that their methods must act in some different way and attempts were made to harmonize the new findings with the theory that parathyroid tetany was due to an intoxication, a theory supported by the experiments of Paton and his associates (1916), W. F. Koch (1912, 1913), and the writings of Biedl (1922). The bread, milk, and lactose diet of Dragstedt and Peacock (1923) was found to check bacterial putrefaction or proteolysis in the intestinal tract and to control the toxemia associated with low intestinal obstruction (Cannon, Dragstedt and Dragstedt, 1920). It was assumed that it controlled the "toxemia" of parathyroid tetany in the same way and that this was therefore due to the absorption of toxic basic amines probably produced by bacterial putrefaction in the intestine. The beneficial effect of the daily repeated intravenous administration of Ringer's solution (Luckhardt and Rosenbloom, 1922) was assumed to be due to the vigorous diuresis produced and the removal of toxic substances by way of the urine.

During the past decade a very large number of observations have been reported which seem to show a consistent relation between the lowering in the concentration of the serum calcium and the symptoms of tetany which follow extirpation of the parathyroid glands. The evidence that this is a causal relationship may be summarized as follows. When tetany appears following thyro-parathyroidectomy the serum calcium is usually reduced from a normal of about 10 mgm. per 100 cc. to below 7, while the concentration of sodium and potassium is unchanged (MacCallum and

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Voegtlin, 1909; Hastings and Murray, 1921; Salvesen, 1923). The extent of lowering of the blood calcium corresponds roughly with the degree of severity of the tetany in recently operated animals. The removal or inactivation of the circulating calcium in normal animals by the intravenous injection of sodium citrate, oxalic acid, or sodium oxalate is accompanied by muscular twitchings and convulsions similar to those of tetany, and these may be relieved by the injection of soluble calcium salts in sufficient quantity to restore the equilibrium (MacCallum and Vogel, 1913; Trendelenburg, 1921). The perfusion of blood from a dog in active tetany into the isolated leg of a normal dog increases the electrical excitability of the nerves in the perfused leg (MacCallum, 1913; Jacobson, 1923) and a similar effect may be obtained by perfusing normal blood from which the calcium has been removed by dialysis (MacCallum, Lambert and Vogel, 1914). The intravenous injection of certain soluble calcium salts relieves for a time all the signs and symptoms of tetany (MacCallum and Voegtlin, 1909). Corresponding to this relief the blood calcium returns to its normal level and on the return of tetany is found again at a low level (Salvesen, 1923). The administration of an active preparation of the parathyroids (Collip, 1925) relieves parathyroid tetany and effects a simultaneous proportionate elevation of the blood calcium. Excessive amounts of this hormone increase the serum calcium above the normal concentration and bring about symptoms of weakness and depression in some respects the antithesis to those of tetany (Collip, 1925; Collip, Clark, and Scott, 1925).

This evidence, coming from many independent sources and with a wide variety of experimental procedure probably justifies the commonly accepted view that the decrease in the serum calcium is the cause of parathyroid tetany. Peters and Van Slyke (1931) state this view as follows: "Tetany may be expected to supervene after parathyroidectomy when the serum calcium falls below 7 mgm. per 100 cc. The rôle of calcium in the etiology of parathyroid tetany, at least, can hardly be questioned longer."

For this reason it was of interest to us to determine if the repeated daily intravenous injection of Ringer's solution would prevent the lowering in the concentration of serum calcium to the extent that it kept the animal free from symptoms of tetany.

The commonly used formulas for Ringer's solution contain calcium in a concentration slightly exceeding that present in normal mammalian serum and it might be supposed that the intravenous injection of large quantities of this solution would prevent the fall in serum calcium after parathyroidectomy. The following experiments were designed to determine this point.

A special pump was designed by one of us (R. R.) to deliver small measured amounts of fluid at a constant rate. It was set to deliver from 1.3 to 2.1 cc. of fluid per minute or from 2000 to 3000 cc. per 24 hours. Seven

adult male dogs were placed on an exclusive lean meat and water diet and after a control blood sample was secured were operated upon under general anesthesia. The thyroid and parathyroid glands were widely excised and the wounds carefully closed. The external jugular vein was then isolated through a small superficial incision. The cephalic end was ligated and a small smooth round rubber catheter tied loosely in the cardiac portion. In some cases this catheter extended along the jugular vein into the right auricle. A close fitting dressing secured the catheter and kept it from being pulled out. It was connected with the pump and the intravenous injection of Ringer's solution started as soon as the animal recovered from the anesthetic. The tube caused little discomfort and was tolerated very well for from 3 to 8 days. Blood samples were secured from time to time and careful watch was made for symptoms of tetany. The electrical excitability of the peripheral nerves was not examined. All of the animals finally died, the causes of death being either tetany, accidental withdrawal of the jugular cannula, erosion of the jugular vein or right auricle, edema of the lungs, or extensive wound infection. The data with respect to the relation between the alternations in the chemistry of the blood and the symptoms of tetany have been summarized in table 1.

The Ringer's solution was made up according to the following formula: NaCl 0.9 gram, $CaCl_2$ 0.024 gram, KCl 0.042 gram, $NaHCO_3$ 0.03 gram, water q.s. ad. 100 cc. Calcium was determined by Clark and Collip (1925) modification of the Kramer and Tisdall method; phosphorus by the method of Fiske and Subbarow (1925); CO_2 by the method of Van Slyke and Neill (1924); pH by the calorimetric method of Hastings (Peters and Van Slyke, 1931); total protein by the macro Kjeldahl method (Peters and Van Slyke, 1931); and the diffusible calcium of the serum by the high pressure ultrafiltration method of Nicholas (1932).

A marked decrease in the concentration of serum calcium occurred in all seven animals whereas an increase in the inorganic phosphate developed in only five. The increase in phosphate was very striking, however, in the two animals that died in tetany. No correlation could be observed between the decrease in the serum calcium and symptoms of tetany. None of the animals, however, survived more than 8 days, most of them dying from accidents due to the injection apparatus connected with the jugular vein rather than to the parathyroid deficiency. Because of this difficulty an attempt was made to control the tetany after thyro-parathyroidectomy in two animals by the injection of large amounts of Ringer's solution at regular intervals during the day. The details of this experiment have been summarized in table 2. In both of these dogs likewise the administration of as much as 3000 cc. of Ringer's solution daily did not prevent the decrease in serum calcium following removal of the parathyroid glands. Tetany was, however, controlled almost entirely and both animals have now sur-

vived the operation for approximately three and one-half months. In each case serum calcium determinations from time to time have revealed values ranging between 4 and 6 mgm. per 100 cc.

DISCUSSION. Normal serum contains approximately 10 mgm. of Ca per 100 cc. A salt solution containing the concentration of phosphate and

TABLE 1

Effect of thyro-parathyroidectomy on the blood chemistry of dogs, treated by the daily intravenous injection of Ringer's solution

DOG NUMBER	WEIGHT	DAYS AFTER OPERATION	REMARKS	RINGER'S SOLUTION PER 24 HRS.	BLOOD CHEMISTRY			
					Ca	P	pH	Total protein
	kgm.			*cc.*	*mgm.*	*mgm.*		*grams*
259	13.1	0	Normal control: Meat diet	0	11.0	5.0	7.48	5.14
		5	No tetany since operation	2000	5.7	4.9	7.48	3.90
272	21.1	0	Normal control: Meat diet	0	10.3	4.3	7.47	4.86
		3	Violent tetany	2000	4.8	7.2	7.30	4.73
		4	No tetany	2000	4.5	6.8	7.46	4.92
		7	No tetany since 3rd day	2000	4.4	8.2	7.41	5.34
307	13.6	0	Normal control: Meat diet	0	10.7	4.2	7.58	3.25
		4	Violent tetany	2000	6.8	5.1	7.36	2.76
		5	No tetany	2000	5.0	6.9	7.45	4.06
		8	No tetany since 4th day	2000	5.8	6.7	7.52	3.82
311	19.7	0	Normal control: Meat diet	0	10.3	5.5	7.46	3.86
		5	No tetany since operation	2000	5.7	6.8	7.53	3.24
		8	No tetany since operation	2000	6.9	5.0	7.53	3.32
319	13.0	0	Normal control: Meat diet	0	10.0	4.6	7.52	5.36
		5	No tetany since operation	2000	5.9	3.7	7.47	3.87
		8	No tetany since operation	2000	5.7	4.5	7.30	3.65
706	12.0	0	Normal control: Stock diet	0	10.2	5.5	7.41	5.12
		3	No tetany since operation	3000	6.8	6.6		
		6	Violent tetany—died in tetany	3000	4.2	12.0	7.32	4.78
489	14.0	0	Normal control: Stock diet	0	11.1	6.8		
		1	No tetany since operation	1000	9.4	7.0		
		3	Violent tetany—died in tetany	3000	5.2	10.8		

bicarbonate found in blood will not carry this amount of calcium in solution. A considerable effort has been expended during the past few years to determine the nature of the serum calcium and the following possibilities have been recognized. Approximately 30 per cent of the total calcium seems to be held in the form of a loose chemical compound with the serum

TABLE 2

Relation of serum calcium to tetany in thyro-parathyroidectomized dogs treated by the intravenous injection of Ringer's solution

DOG NUMBER	DATE (1933)	REMARKS	CALCIUM		P	pH	Total protein
			Serum	Ultrafiltrate			
			mgm.	*mgm.*	*mgm.*		*grams*
882 Adult female Weight 12.32 kgm.	1–11	Diet bread, milk, water ad lib. 9:00 a.m. Blood drawn for chemistry 2:00 p.m. Complete thyro-parathyroidectomy 4:00 p.m. 1000 cc. Ringer's solution intravenously	9.9	7.1	3.9	7.52	5 8
	1–12	8:30 a.m. Condition good. No tetany. Blood sample 1000 cc. Ringer's solution intravenously at 9:00 a.m., 1:00 p.m., and 5:00 p.m.	8.2		4.0		
	1–13	8:30 a.m. Condition good. No tetany. 1000 cc. Ringer's 9:00 a.m. Blood drawn for chemistry 1000 cc. Ringer's at 1:00 p.m. and 5:00 p.m.	8.6		2.8		
	1–14	8:30 a.m. Condition good. No tetany. Blood drawn 9:00 a.m. 1000 cc. Ringer's solution 12:00 Noon. 25 grams Ca lactate —25 grams lactose by stomach tube	7.1	5.0	4.1	7.53	5.3
	1–15	9:00 a.m. Condition good. No tetany. 25 grams of calcium lactate—25 grams lactose by tube					
	1–16	8:30 a.m. Condition good. No tetany. 1000 cc. Ringer's 9:00 a.m. Blood drawn for chemistry 1000 cc. Ringer's at 1:00 p.m. and 5:00 p.m.	8.4		3.4		

TABLE 2—*Continued*

DOG NUMBER	DATE (1933)	REMARKS	CALCIUM		P	pH	Total protein
			Serum	Ultrafiltrate			
			mgm.	*mgm.*	*mgm.*		*grams*
882 Adult female Weight 12.32 kgm.	1–17	Condition good. No tetany. 1000 cc. of Ringer's solution at 8:30 a.m., 1:00 p.m. and 5:00 p.m.					
	1–18	Condition good. No tetany 1000 cc. of Ringer's solution at at 8:30 a.m., 1:00 p.m. and 5:00 p.m.					
	1–19	8:30 a.m. Condition good. No tetany. Blood drawn. Used for other experiment	6.1	5.1	5.5	7.50	5.1
	1–11	Diet bread, milk, water ad lib. 9:00 a.m. Blood drawn for chemistry 2:30 p.m. Complete thyro-parathyroidectomy 4:30 p.m. 1000 cc. Ringer's solution intravenously	10.0	6.5	3.7	7.54	5.8
	1–12	8:00 a.m. Condition good. No tetany. Blood sample 1000 cc. Ringer's at 9:00 a.m., 1:00 p.m. and 5:00 p.m.	8.0		4.2		
766 Adult female Weight 7.73 kgm.	1–13	8:30 a.m. Condition good. No tetany. 1000 cc. Ringer's 9:00 a.m. Blood drawn for chemistry 1000 cc. Ringer's at 1:00 p.m. and 5:00 p.m.	8.1		3.1		
	1–14	8:30 a.m. Condition good. No tetany. Blood drawn 9:00 a.m. 1000 cc. Ringer's solution 1:00 p.m. 25 grams Ca lactate—25 grams lactose by mouth	5.6	3.3	5.1	7.53	5.3
	1–15	8:30 a.m. Condition fair. No tetany. Loss of appetite 25 grams calcium lactate—25 grams lactose by tube					

TABLE 2—*Concluded*

DOG NUMBER	DATE (1933)	REMARKS	CALCIUM		P	pH	Total protein
			Serum	Ultrafiltrate			
			mgm.	*mgm.*	*mgm.*		*grams*
776 Adult female Weight 7.73 kgm.	1–16	8:30 a.m. Condition good. No tetany. 1000 cc. Ringer's 9:00 a.m. Blood drawn 1000 cc. Ringer's at 1:00 p.m. and 5:00 p.m.	6.9		3.7		
	1–17	Condition good. No tetany. 1000 cc. Ringer's solution at 9:00 a.m., 1:00 p.m. and 5:00 p.m.					
	1–18	8:30 a.m. Tremors of temporal muscles. Blood drawn 1000 cc. Ringer's at 9:00 a.m., 1:00 p.m. and 5:00 p.m.	4.7	3.9	5.2	7.49	4.9
	1–19	8:30 a.m. Tremors of temporal muscles. Blood drawn. Used for other experiments	3.9		6.2		

proteins. If serum is filtered through a cellophane membrane which holds back the proteins the calcium in the filtrate is reduced to about 7 mgm. per 100 cc. For convenience we shall express the fraction of calcium held in combination with the serum proteins as the A fraction. In normal animals it amounts to about 3 mgm. per 100 cc. and is assumed to be physiologically inert because the hypocalcemia associated with the low serum proteins in nephritis and sprue does not provoke tetany. Of the 7 mgm. of calcium in the protein free ultrafiltrate it has been estimated that about 2 mgm. may be accounted for by the forces which ordinarily govern solubility in salt solutions. This moiety we have designated as fraction C. Five milligrams or roughly one-half of the total calcium is assumed to be held in solution by forces dependent directly or indirectly on the physiological activity of the parathyroid glands. This we have called fraction B. The fact that complete removal of the parathyroid glands lowers the serum calcium about 50 per cent has been thought to support this view. We have pointed out elsewhere, however, that the serum calcium of parathyroidectomized dogs may be restored to normal levels by the oral administration of calcium lactate without supplying any parathyroid product. It is furthermore assumed that this B fraction is physiologically active and that its lowering after parathyroidectomy causes tetany and failure of calcification of osteoid tissue, and its increase following parathormone administra-

TABLE 3

Experiments illustrating the influence of varying concentrations of calcium on the spontaneous activity of frog muscle and nerve-muscle preparations suspended in calcium-free Ringer's solution and in 0.7 per cent NaCl solution

DATE (1933)	TIME	PREPARATION NUMBER	TYPE OF PREPARATION	SOLUTION	TEMPERATURE OF SOLUTION	RESPONSE
					°C.	
1–11	11:00	1	Frog gastrocnemius muscle and nerve	B	20	No contractions in 20 minutes
	11:10	2	Frog gastrocnemius muscle and nerve	A	20	No contractions in 10 minutes
	11:20	1	Frog gastrocnemius muscle and nerve	A	20	No contractions in 10 minutes
	11:20	2	Frog gastrocnemius muscle and nerve	B	20	No contractions in 10 minutes
	11:30	1	Frog gastrocnemius muscle and nerve	A	38	No contractions in 10 minutes
	11:30	2	Frog gastrocnemius muscle and nerve	B	38	No contractions in 10 minutes
1–12	10:00	3	Frog gastrocnemius muscle and nerve	B	20	No contractions in 5 minutes
	10:05	3	Frog gastrocnemius muscle and nerve	C	20	Vigorous rhythmical contractions
	10:10	3	Frog gastrocnemius muscle and nerve	B	20	Contractions ceased in 15 seconds
	10:15	3	Frog gastrocnemius muscle and nerve	C	20	Contractions began in 10 seconds
	11:30	4	Frog sartorius	C	20	Contractions began in 2 seconds
	11:30	5	Frog sartorius	C	20	Contractions began in 2 seconds
	11:31	4	Frog sartorius	C + 1 mgm. Ca	20	Contractions ceased in 1 minute
	11:31	5	Frog sartorius	C + 1 mgm. Ca	20	Contractions ceased in 4 minutes
	11:32	4	Frog sartorius	C	20	Contractions began in 10 seconds
	11:36	5	Frog sartorius	C	20	Contractions began in 5 seconds
	11:39	4 and 5	Frog sartorius	C + 1 mgm. Ca	20	Contractions ceased in 1 minute
	11:41	4 and 5	Frog sartorius	B	20	No spontaneous contractions
	11:45	4 and 5	Frog sartorius	C + 1 mgm. Ca	20	No spontaneous contractions
	11:47	4 and 5	Frog sartorius	C	20	Muscle 4 no contractions Muscle 5 contractions began in 10 seconds
	1:00	6	Frog sartorius	B	20	No spontaneous contractions
	1:03	6	Frog sartorius	A	20	No spontaneous contractions in 6 minutes

1–12	1:09	6	Frog sartorius	C	20	Contractions began in 5 seconds
	1:10	6	Frog sartorius	C + 1 mgm. Ca	20	Contractions ceased in 10 seconds
	1:11	6	Frog sartorius	C	20	Contractions began in 5 seconds
	1:13	6	Frog sartorius	B	20	Contractions ceased in 10 seconds
	1:24	6	Frog sartorius	C + 1 mgm. Ca	20	No contractions in 4 minutes
	1:28	6	Frog sartorius	C	20	Contractions began in 5 seconds
1–13	2:13	7	Frog sartorius	B	20	Feeble contractions which ceased in 5 seconds
	2:16	7	Frog sartorius	C + 0.5 mgm. Ca	20	Contractions began in 5 seconds
	2:18	7	Frog sartorius	B	20	Contractions ceased in 10 seconds
	2:20	7	Frog sartorius	C + 1.0 mgm. Ca	20	Feeble contractions began in 5 seconds
	2:23	7	Frog sartorius	B	20	Contractions ceased in 15 seconds
	2:25	7	Frog sartorius	A	20	No contractions appeared
	2:28	7	Frog sartorius	C + 1.0 mgm. Ca	20	Contractions began in 6 seconds
	2:29	7	Frog sartorius	B	20	Contractions ceased in 12 seconds
	2:31	7	Frog sartorius	C + 1.5 mgm. Ca	20	Feeble contractions began in 1 minute
	2:33	7	Frog sartorius	A	20	Contractions ceased in 15 seconds
	2:35	7	Frog sartorius	C + 1.5 mgm. Ca	20	Feeble contractions began in 1 minute
	3:08	8	Frog sartorius	B	20	No contractions
	3:10	8	Frog sartorius	C + 2 mgm. Ca	20	Very feeble contractions began in 20 seconds
	3:12	8	Frog sartorius	B	20	Contractions ceased in 10 seconds
	3:14	8	Frog sartorius	A	20	No contractions
	3:16	8	Frog sartorius	C + 2 mgm. Ca	20	Slight contractions began in 30 seconds
	3:17	8	Frog sartorius	B	20	Contractions ceased
5–26		9	Frog sartorius + gastrocnemius	A	20	No contractions in 24 hours
		10	Frog sartorius + gastrocnemius	B	20	No contractions in 24 hours
		11	Frog sartorius + gastrocnemius	C	20	Repeated rhythmical contractions for 6 hours
		12	Frog sartorius + gastrocnemius	D	20	No contractions in 12 hours

Note: The solutions were made up as follows: Solution A, NaCl 152, KCl 5, and $MgCl_2$ 3 m. eq. per l., pH 9.0; solution B, NaCl 148, $CaCl_2$ 3.5, KCl 5.0, $MgCl_2$ 3.0 m. eq. per l., pH 8.0; solution C, NaCl 120 m. eq. per l., pH 7.5. (In several instances from 1 to 2 mgm. of Ca as $CaCl_2$ per 100 cc. were added to solution C.) Solution D, NaCl 155, KCl 5 m. eq. per l.

tion causes marked depression. There remains the probability that a portion of the total calcium is present in the form of calcium ions. The work of Ringer, J. Loeb, R. S. Lillie, and others suggests that it is this form of calcium that is physiologically active at least so far as effects on the irritability of muscles and nerves are concerned. We may designate this ionic calcium as fraction D. All of these various fractions are in equilibrium since R. Loeb (1924) has shown that if serum be dialyzed against 0.85 per cent NaCl all of the calcium will be removed. Each of the various fractions are thus potential sources for ionic calcium. This ionic Ca probably exists in relatively low concentration in normal serum. Many attempts have been made to calculate its probable concentration but no accurate methods have been found to measure it directly.

R. S. Lillie (1923) has recently summarized our knowledge with respect to the influence of the ions in the surrounding medium on the irritability of muscle and nerve, pointing out that the ions Na, K, and Ca exert a surface action of the plasma membrane. A definite concentration of sodium ions seems to be necessary for the maintenance of the normal irritability of these structures. The stimulating action of sodium is antagonized by calcium ions as well as by certain other metals such as magnesium. If the sartorius muscle of a frog be placed in pure 0.7 per cent NaCl solution it promptly displays a series of continuous contractions and irregular twitchings. If a small amount of a soluble calcium salt be added the contractions cease, an observation of J. Loeb (1899) that we have recently confirmed. We have furthermore attempted to determine the minimum amount of calcium necessary to abolish the stimulating action of sodium in a series of experiments summarized in table 3. The addition of only 1 to 2 mgm. of calcium as $CaCl_2$ per 100 cc. was found sufficient to abolish the spontaneous contractions of the frog sartorius muscle immersed in 0.7 per cent NaCl solution. This calcium is presumably chiefly ionized. To our considerable surprise we found that whereas isolated muscles or nerve-muscle preparations placed in a water solution of pure 0.7 per cent NaCl displayed spontaneous rhythmical contractions, they remained quiescent if immersed in solutions (sols A and D) containing the other salts of the serum but no calcium. The small amounts of magnesium (3 m. eq. per l.) and of potassium (5 m. eq. per l.) in normal plasma seem to be sufficient to counterbalance the effect of the absence of calcium at least so far as this type of experiment is concerned. The fact that removal of the parathyroid glands does not alter the concentration of potassium and magnesium in the blood thus presents a difficulty to the calcium deficiency theory of tetany, that deserves further study.

It may be of significance in this connection to record the following experiment. The gastrocnemius and sartorius muscles of a frog were placed in 25 cc. of solution A (calcium-free Ringer's solution) and in an equal volume

of solution B (Ringer's solution) at room temperature. The muscles in both solutions remained quiescent. After 30 minutes 5.0 cc. of 1.0 per cent sodium citrate were added to each flask. Within a few minutes the muscles in solution A began to contract rhythmically while those in B remained quiet.

The relief and control of tetany obtained in these experiments by the intravenous injection of Ringer's solution may still be harmonized with the theory that a diminution in the serum calcium is the immediate cause of parathyroid tetany. It would seem to be necessary to assume, however, that it is only the ionic calcium that is physiologically active and that the administration of Ringer's solution maintains this fraction at an effective concentration in spite of a fall in the total calcium in some instances as great as 50 per cent. There remains however a considerable difficulty in understanding how the removal of the parathyroid glands can reduce the ionic calcium to a sufficient degree to cause tetany unless we postulate the accumulation in the blood of these animals of some substance which acts like citrate in inactivating calcium. It is possible that guanidine and similar products may play such a rôle.

SUMMARY

1. The continuous intravenous injection of Ringer's solution in amounts of from 2000 to 3000 cc. per 24 hours was found to prevent the onset of tetany after complete thyro-parathyroidectomy in dogs but did not prevent the usual diminution in the serum calcium.

2. Frog muscles suspended in 0.7 per cent NaCl solution displayed spontaneous rhythmic contractions which could be abolished by the addition of as little as 1 to 2 mgm. of Ca per 100 cc. of solution. Such muscles suspended in physiological salt solutions containing all of the inorganic salts of the serum but no calcium displayed no spontaneous activity.

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STUDIES ON THE RESPIRATION OF ANIMAL TISSUES[1]

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The utilization of oxygen by living cells is a complex mechanism involving the chemical reactions necessary for the oxidation of the organic metabolites. The fundamental problem concerning all biological oxidations is an explanation of the fact that carbohydrates, fatty acids, and other organic metabolites are readily oxidized at ordinary temperatures when in the presence of the living cell, but remain fairly stable when in contact with atmospheric O_2 alone.

Through the work of Schönbein (1), Bach (2), Wieland (3), Thunberg (4), Kastle (5), Dixon (6), and others, it has been shown that there is present in tissue cells a number of enzyme catalysts of oxidation (oxidases) which are together responsible for tissue respiration. These oxidases have been subdivided according to the mechanism of their action into those which activate the metabolites, dehydrogenases (anaerobic and aerobic), and those that activate oxygen. In contrast to the fact that there exists in tissues a number of oxidases which are together responsible for cellular respiration is Warburg's (7) theory of cell respiration which has assumed considerable prominence during the last decade. According to Warburg's theory tissue respiration is to be regarded as being due to the action of one catalytic iron containing system, rather than to the sum-total of a number of oxidations brought about by several catalysts.

The recent work of Keilin (8) has done much to clear up the various types of oxidase systems. He has shown that at least one oxidizing system consists of a specific substrate with its dehydrogenase, an oxygen carrier, cytochrome, and an oxygen activator, indophenol oxidase. He has obtained convincing experimental evidence showing that the oxygen activating enzyme of the cytochrome system is identical with Warburg's respiratory enzyme. More recently Cook, Haldane, and Mapson (9) propose giving the name "oxygenase" to the cell catalyst which activates molecular oxygen; thus maintaining the nomenclature of naming enzymes by adding an "ase" to the substrate activated.

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Little experimental work has been done on the total respiration and the rate of respiration of various animal tissues. The oxygen uptake of each tissue constitutes a measure of the sum total of the activity of the various individual oxidase systems. A decrease from the normal in oxygen consumption is therefore due to injury to the enzymatic systems or to a limited supply of substrate. If a plentiful amount of substrate is provided it should be possible, by measuring the total oxygen uptake, to observe the effect of pathological changes on the activity of individual enzyme systems. Likewise a relative value of the metabolic activity of individual tissues may be obtained by a study of their oxygen uptake. In this paper is presented a study of the oxygen uptake of various animal tissues from normal and abnormal animals and the effect of cyanide on the oxygen consumption.

METHOD. A manometric method involving the use of the Barcroft differential respirometer was used for the measurement of the oxygen uptake.

The respirometers were calibrated by attaching the flasks to the manometer and connecting one end of the manometer to a calibrated 1 cc. pipette filled with mercury. The entire system is brought to a temperature and pressure equilibrium. Then by slightly increasing the height of the mercury in the pipette a displacement in the manometer is produced. The linear displacement of the manometer reading is readily converted into volume by dividing the cubic millimeters of mercury displaced in the pipette by the change in millimeters of the manometer level. The factor thus obtained may be converted according to the pressure and temperature condition under which the experimentation is conducted. For more complete details regarding the calibration of the apparatus the papers of Hoffman (10), Munser and Neuman (11), or Stephenson (12) may be consulted.

In using the Barcroft apparatus to measure the oxygen uptake of tissue preparations, it is, of course, necessary that the effect of the CO_2 produced by the tissue be eliminated. This is accomplished by the technique described by Dixon and Elliott (13), which consists of placing in the small open tube sealed to the bottom of the flask a small piece of filter paper, approximately 2 cm. square, rolled into a cylinder and frayed at the upper end. This paper when moistened with 6 per cent NaOH very efficiently absorbs the CO_2 produced.

The technique employed was as follows: differential Barcroft microrespirometers were used in each experiment. Into each of the flasks were pipetted 2.8 cc. of buffer solution (pH 7.3) and into the right hand flask of each apparatus was weighed approximately 0.2 gram of the tissue (the preparation of which is described later). Into the left hand (compensation) flasks were pipetted 0.2 cc. of distilled water. The small tubes in all of the respirometer flasks contained rolls of filter-paper moistened with 6

per cent NaOH. The respirometers were then placed in a constant temperature bath (adjusted to 37.2° ±0.01) and agitated by the shaking mechanism. After allowing ten minutes for equilibration, the taps of the apparatus were closed and readings taken every 20 minutes for the next hour. The addition of any other substance to the respiration flask, for example KCN, was always made to both the right and left flasks and was compensated for by withholding an equivalent volume of buffer solution, so that the total volume was always 3 cc.

The buffer used in all cases, except where otherwise stated, consisted of M/30 NaH_2PO_4 containing 0.2 per cent glucose and adjusted to a pH 7.3 with NaOH. In the experiments with cyanide, 0.3 cc. of N/10 KCN was added which gave for 3 cc. a cyanide concentration of M/100. The cyanide solution was adjusted so as not to affect the pH of the tissue medium.

The tissues were used immediately after the animal was killed, and usually within 12 to 15 minutes after the death of the animal the stop cocks of the respirometers were closed and observations recorded. It should be noted that the tissues were used just as taken from the animal, they were not washed or extracted and the only treatment they received was the slicing or mincing according to the form in which they were used. Quite obviously the condition of the tissue would affect the oxygen consumption, that is, the thinness of the individual tissue fragments used must be such that the oxygen may readily be available to all of the tissue cells, not just those cells at the periphery. Warburg (14) recommends tissues sliced with a razor moistened with Ringer's solution into slices 0.5 mm. or less in thickness. Dixon and Elliott minced the tissue in the form of a fine suspension; the mincing was not so fine as to involve excessive injury to the tissue cells. They observed no difference in the rate of respiration or the inhibition produced by cyanide in comparative experiments on thinly slicing the tissue or mincing the tissue to a thick homogeneous paste. We likewise observed no difference in comparative experiments in which tissues were sliced very thin with a razor and in which the tissue was finely minced with a scissors. In the experiments reported in this paper the tissues were prepared according to the latter method. An amount of this tissue mince was transferred to the respirometer flask, weighed, and the oxygen uptake recorded.

Duplicate or triplicate determinations carried out on different samples of the same tissue usually agreed quite closely. However, comparing different tissues considerable variation in the rate of respiration was observed, even with corresponding tissues from different individuals of similar age and nutrition. Table 1 gives typical values and illustrates the accuracy of the method.

The results may be conveniently represented in the form of curves in which cubic millimeters of oxygen uptake are plotted against time. Chart

1 shows typical results plotted. These curves are all essentially of the same form and show that during the first thirty to forty minutes the oxygen uptake is almost linear, but after that interval the rate of respiration decreases slightly.

For satisfactory experimentation an accurate and finely adjusted thermostatic control of the constant temperature bath is essential. Differences in temperature markedly affect the rate of the oxygen uptake process. For example, the average oxygen uptake in cubic millimeters per gram per

TABLE 1

Typical results obtained

TISSUE	ANIMAL	O_2 UPTAKE	ERROR	O_2 UPTAKE IN M/100 KCN	ERROR
		cmm./gr./hour	*per cent*		*per cent*
Liver	Normal chick	1162 1240	6.0		
	A-deficient chick	1285 1200 1260 1220	0.5		
	Pellagra chick	1405 1410	0.4	514 580	3
Kidney	Normal chick	1206 1224 1220	0.4		
	B_1-deficient chick	1370 1260	8.5		
Cerebrum	Normal chick	1105 1176	5.9	478 418	12
Liver	Normal rat	660 666	1.0		

hour for normal chick liver at 37.2° is 1226, whereas the value for the same material measured at 20° is 311. For the livers of chicks deficient in vitamin A the average oxygen uptake is 1014 c.mm. per gram per hour measured at 37.2° and 278 c.mm. measured at 20°. Fluctuation of as little as a tenth of a degree will produce a slight change in the manometer levels.

EXPERIMENTAL AND DISCUSSION. The results for the rate of respiration of the tissues studied are given in table 2. It is observed that in the chick the rates of oxygen uptake for the liver, kidney, and brain tissue are all in

the region of 1000 to 1500 c. mm. per gram per hour, and that for heart and muscle tissue the values run from 200 to 500 c.mm. per gram per hour.

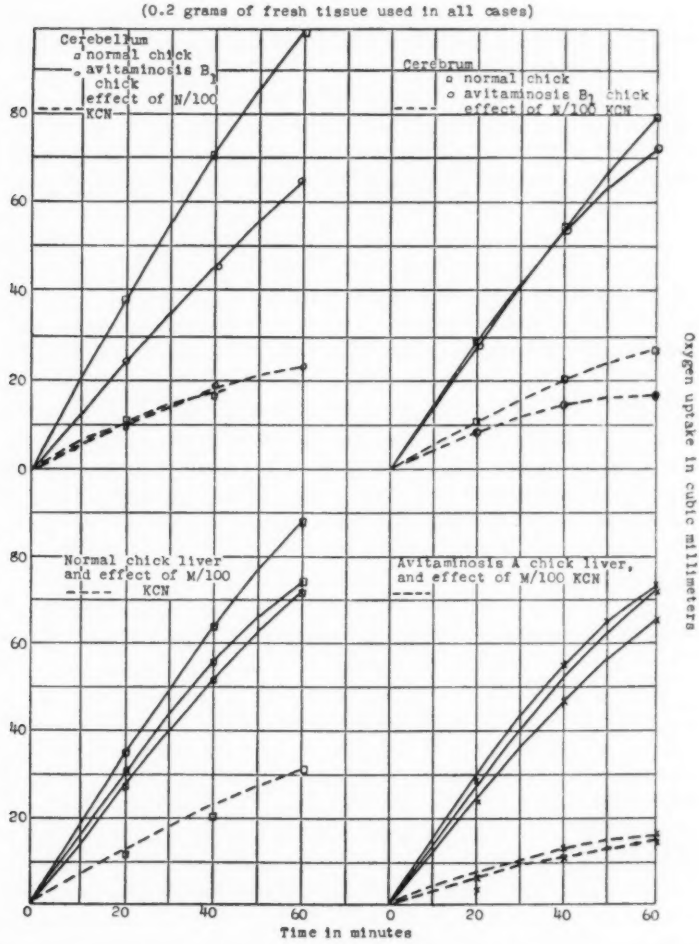

Chart 1. Typical curves for the rate of oxygen uptake of various tissues in glucose phosphate buffer, with and without cyanide.

This shows that muscle tissue, whether cardiac or striated, has a much lower metabolic activity than the other tissues studied. From experiments made upon tissues from rats it is observed that muscle tissue has a lower

rate of oxygen uptake than has liver tissue. The average values in cubic millimeters per gram per hour for the tissues studied in the normal rat are liver 887, testis 751, and muscle 623. It is observed that the oxygen uptake values of liver and muscle from anemic rats is practically the same as the results from the normal animals. With the exception of normal rat liver

TABLE 2

Oxygen uptake of animal tissues

All values for oxygen uptake are reported in cubic millimeters per gram of fresh tissue per hour. Cyanide inhibition is reported as per cent of the normal.

	NUMBER OF DETERMINATIONS	RATE OF OXYGEN UPTAKE			INHIBITION PRODUCED BY M/100 KCN
		Maximum	Minimum	Average	
Normal chick liver	9	1,140	1,340	1,226	62
A-deficient chick liver	10	779	1,366	1,014	70
B_1-deficient chick liver	7	680	1,870	1,197	
B_2-deficient chick liver	3	1,350	1,407	1,372	67
Normal chick kidney	5	1,200	1,700	1,442	72
A-deficient chick kidney	5	830	1,610	1,218	67
B_1-deficient chick kidney	6	543	2,040	1,210	
Normal chick cerebrum	7	854	1,360	1,145	65
A deficient chick cerebrum	2	1,125	1,260	1,193	59
B_1-deficient chick cerebrum	5	1,010	1,300	1,186	69
Normal chick optic lobe	6	1,120	1,320	1,203	67
B_1 deficient chick optic lobe	4	1,064	2,230	1,472	
Normal chick remainder	4	896	1,035	963	58
B_1-deficient chick remainder	3	735	1,030	959	
Normal chick cerebellum	6	1,050	2,140	1,486	63
A-deficient chick cerebellum	2	956	1,050	1,003	42
B_1-deficient chick cerebellum	5	510	1,510	959	48
Normal chick heart	4	304	380	325	
B_1-deficient chick heart	6	229	510	359	
B_1-deficient chick muscle	6	179	510	319	
Normal rat liver	19	663	1,072	894	60
Anemic rat liver	10	663	1,050	857	52
Normal rat muscle	4	348	857	623	
Anemic rat muscle	1			590	
Normal rat testis	4	720	780	751	

and muscle the values reported in table 2 are the only ones, so far as is known, to appear in the literature.

Comparing the average values obtained there seems to be no significant difference between the rate of oxygen uptake of the livers of normal chicks, vitamin A deficient chicks, vitamin B_1 deficient chicks, and vitamin B_2 deficient chicks. They all have a rate of oxygen uptake approximating 1200 c.mm. per gram per hour. Likewise in the case of the oxygen uptake

of kidney, heart, and muscle, the values of which approximate 1300, 400, and 300 c.mm. per gram per hour respectively, there seems to be no marked difference between normal chicks and chicks deficient in certain of the vitamins. There is, as can readily be ascertained from table 2, a distinct difference in the rate of respiration of individual tissues.

Considering now the results obtained with cyanide, it will be seen from table 2 that cyanide in the concentration used (M/100) produces a marked, but by no means complete, inhibition of respiration; usually it inhibits between 60 and 70 per cent or roughly two-thirds of the total respiration. Dixon and Elliott made quite a thorough study of the effect of cyanide on the respiration of tissues. They report that in most cases a cyanide concentration of M/1000 is sufficient to produce the maximum degree of inhibition obtainable, and that increasing the cyanide concentration as much as 100 times gives no greater inhibition. These experiments reported with a cyanide concentration of M/100 thus represent maximum cyanide inhibition. The data obtained in the cyanide experiments support those of Dixon and Elliott and clearly show that approximately one-third of the total respiration is due to systems not poisoned by cyanide; therefore the oxygenase system can only account at most for about two-thirds of the total respiration of typical animal tissues.

Because Elvehjem and Neu (15) have shown that the uric acid content of the blood of the chick is increased in avitaminosis A, it was thought advisable to investigate the xanthine oxidase activity of the livers of normal and vitamin A deficient chicks. This was done by extracting the xanthine oxidase from the fresh livers with 10 volumes of 2 per cent sodium fluoride. Two cubic centimeters of this extract were placed in each flask of the Barcroft manometer and to the right hand flask was added substrate in the form of acetaldehyde. The substrate solution was tested previously in the Barcroft apparatus, using fresh whole cow's milk to supply the xanthine oxidase, and found to be readily oxidized. Xanthine was not used as a substrate because at a pH 7.3 it is difficult to keep the xanthine in solution. No significant difference with acetaldehyde as the substrate was noted in the xanthine oxidase activity of the normal and vitamin A deficient liver. For example, at 37.2°, 2 cc. of extract from a normal chick liver registered an oxygen uptake of 27.1 c.mm. per hour as compared to 27.2 c.mm. for the extract from the liver of the avitaminosis A chick. Further experiments using xanthine and hypoxanthine as substrates should be conducted in studying the xanthine oxidase activity of the liver of normal and vitamin A deficient chicks.

In experiments on brain tissue, the brain was divided into four parts and each part observed separately. These divisions of the brain were the cerebellum, cerebrum, optic lobes, and the rest of the brain remaining was termed the "remainder."

The oxygen uptake of brain tissue in vitro has been investigated probably more extensively than any other tissue. Loebel (16), Meyerhof and Lohmann (17), Warburg, Posener and Negelein (18) and Holmes (19) have found in rat and frog brain rather large and variable oxygen uptakes lasting for several hours, which could be maintained at approximately initial intensity for some time by addition of glucose and lactate to the Ringer's solution in which the tissue was suspended. Abderhalden and Schmidt (20), Roche (21), Dickens and Simer (22), and Quastel and Wheatley (23) also have studied oxidation processes in the brain.

Several attempts have been made to correlate the symptoms of vitamin B deficiency with faulty oxidations in the body and more particularly to oxidations in the brain tissue. The early work was before the differentiation of the vitamin B complex, but insofar as the tissues of polyneuritic pigeons were used it is probable that the work has been actually performed on the vitamin B_1 constituent of the complex. Dutcher (24) and Findlay (25) reported a fall in catalase and glyoxalase content respectively in certain tissues of the bodies of pigeons deficient in vitamin B_1, and found that the lost enzymatic power was not restored by the addition of yeast extracts in vitro. The studies of Abderhalden with Schmidt (20), and with Wertheimer (26) upon the oxygen uptake of tissues, Hess (27), and Hess and Messerle (28) upon the reducing properties of tissues for dyestuffs believed that a vitamin B_1 deficiency produced a decrease in the intensity of tissue oxidation. Terroine and Roche (29), Roche (30), and Marrian and Drummond (31) obtained no evidence in support of a failure of tissue oxidation in vitamin B_1 deficiency. Peters and associates have presented an interesting series of papers (32) which seem to show that a deficiency in vitamin B_1 definitely lowers the oxygen uptake in certain localized sections of the pigeon brain. They have found an increase in the lactic acid content of the lower parts of the brains (optic lobe and remainder) of vitamin B_1 deficient pigeons, suggesting an oxidative enzyme deficiency. They have demonstrated that pigeons deficient in vitamin B_1 show a lower oxygen uptake in vitro in the lower parts of the brain. The lowering is not general, as they found no such change in the cerebellum of the pigeon. This decreased power of oxygen consumption in vitro is apparent in a buffer medium containing glucose, lactate, or pyruvate, and to a goodly extent this lowered oxygen uptake is lessened by addition of vitamin B_1 extract (torulin) in vitro, or by the cure of the bird. In a plain phosphate buffer or in phosphate buffer plus succinate as the substrate, no lowering of oxygen uptake was observed. In pigeons which have been dosed with vitamin B_1, but in which no increase in weight has taken place, the tissue shows a power of oxidation approximating the normal, thus showing that the depression of oxidation is not merely associated with the state of nutrition as judged by the weight, but is some expression of the avitaminosis.

It would be interesting to observe avitaminosis B_1 in relation to the oxygen uptake of the chick brain. Protocols for measurement of the oxygen consumption of brain tissue from chicks are given in table 2. The experiments were conducted at 37.2° in a phosphate buffer pH 7.3 with 0.2 per cent glucose as substrate. It is seen that the cerebrum of normal and vitamin B_1 deficient chicks does not show a significant difference in the rate of oxygen consumption. The average values in cubic millimeters per gram of tissue per hour are 1145 and 1186 respectively. Likewise for the optic lobes and remainder there is no marked difference in oxygen utilization between normal and vitamin B_1 deficient chicks, their respective average values being 1203 and 963 for the normal chicks and 1472 and 959 for the avitaminosis chicks. The oxygen uptake of the cerebellum presents some difficulties and at present we are unable to report definitely. It seems that a deficiency of one of the factors of the vitamin B complex markedly affects the oxygen uptake of the cerebellum although we are of the opinion that a factor other than B_1 is involved.

Cyanide inhibition experiments approximate 60 per cent inhibition for all divisions of the brain in both the normal and avitaminosis chicks. A few experiments, the protocols of which are not reported, were carried out in phosphate-Ringer buffer using lactate as a substrate and in some cases adding methylene blue to serve as an oxygen carrier. In general these experiments support the data given in table 2.

SUMMARY

Values obtained for the oxygen uptake as determined with the Barcroft respirometer are given for various tissues of chicks and rats in different nutritional conditions. Data are presented to show the close agreement between duplicate and triplicate values determined on the same tissue.

In normal chicks the rates of oxygen uptake for liver, kidney and brain tissue are all in the region of 1000 to 1500 c.mm. per gram of fresh material per hour. Muscle, both cardiac and striated, has a considerably lower oxygen requirement with values ranging from 230 to 380 c.mm.

With the exception of the cerebellum the oxygen uptake of tissues from chicks deficient in certain of the vitamins shows little difference from those of the normal. Likewise there is no significant difference in the oxygen uptake of the livers of normal and anemic rats.

The maximum cyanide inhibition varies in different tissues between 48 and 87 per cent, the average being about 60 per cent. Support is thus given to the findings of Dixon and Elliott that the respiration of animal tissues is made up of two parts. One, accounting for about two-thirds of the total, is due to enzyme systems poisoned by cyanide; the other one-third is due to systems which are stable to cyanide.

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THE VARIATIONS OF INTRAGASTRIC TEMPERATURE IN RESPONSE TO VASODILATING AGENTS

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The study of intragastric temperature is a relatively recent one, due mainly to lack of a suitable method of investigation in the past. Reports of such studies, carried out only as recently as the beginning of the century, are confusing. Accordingly it was believed that a study of intragastric temperature and effect on it of vasodilating drugs and gastric secretogogues, might throw light on some physiologic processes of the stomach.

The importance of vascular changes in relation to cutaneous temperature has been carefully worked out by Benedict, Brown and others. Whether their findings in this respect hold true with regard to the temperature of an internal organ is difficult to say. It seems that even if the volume of blood in the vessels of the stomach were increased two or three times by vasodilatation, the gastric temperature might still be the same. To judge, therefore, the degree of vasodilatation of the gastric vessels by means of intragastric studies of temperature may not be possible. The possibility of interrelationship of changes in intragastric temperature secondary to changes in blood supply of the stomach cannot be entirely disregarded, however.

METHOD AND MATERIAL. The apparatus used in the experiments to be described was made under the direction of Dr. Charles Sheard and consisted of a small thermocouple fitted into the end of a small-sized Rehfuss tube. The temperature was registered on a mirror galvanometer. The tip of the tube was covered with shellac to prevent erosion of wires by the gastric acids.

A similar Rehfuss tube, for aspiration of gastric content, was then tied parallel with the tube containing the thermocouple. This arrangement allowed simultaneous determination of the temperature and gastric acids at any point in the stomach. The joined tubes were inserted comparatively similar distances in all subjects for all readings. Oral, rectal and dermal temperatures were taken at the same intervals as the other determinations, the mercury thermometers being standardized by comparison with the thermocouple readings. The room temperature in all the experiments was practically identical. The aspirated gastric content was exam-

ined for free acid, total acid, and total content of chloride. Readings were taken when the patient was fasting and at intervals of five, fifteen, thirty, sixty, ninety, and one hundred twenty minutes after administration of the drug or test meal. When the drug was given orally, the liquid was heated to the temperature of the fasting stomach in that particular case. The results following the administration of only histamine, acetylcholine, and alcohol will be considered in detail, since other drugs tried gave no appreciable reaction.

All of the procedures, except those in which typhoid vaccine was used, were carried out approximately from 6 to 8 o'clock in the morning. The subjects as far as possible were in the same physiologic state, most of them being awakened from their night's sleep. All of the subjects had either peptic ulcers, previously definitely diagnosed, or had symptoms of ulcer sufficient to cause them to be in the hospital for observation and medical treatment.

The experiments were controlled by observing under similar conditions a group of ten subjects with similar diagnoses, who did not receive any drugs (or any test meal).

The results of the experiments were evaluated statistically. The change in temperature was calculated from the fasting value of each patient. The difference at each time interval was averaged for all the experiments and the probable error of these calculated.

HISTAMINE. Thirteen experiments with histamine phosphate were carried out, using a dosage of 0.1 mgm. for each kilogram of body weight, given subcutaneously.

Temperature. In all the experiments the actual rectal temperature was higher than the oral temperature, and the intragastric temperature was higher than the rectal temperature. The oral and rectal temperatures rose steadily over the two hours following the injection of histamine; the maximal average rise in oral temperature was 0.25°C.; for the rectal temperature it was 0.19°C. The maximal average rise of forehead temperature was 1.92°C.

The intragastric temperature fell an average of 0.18°C. immediately following the injection of histamine. While the mean fall is not significant statistically, the fact that in eleven of thirteen subjects there was a fall before a rise indicates that this fact is probably significant. Following this the temperature rose at a rate comparable with that of the oral and rectal temperatures. To account for the immediate fall and subsequent slow rise, Brown suggests that after an initial vasoconstriction of the gastric vessels, there is a slow moderate vasodilatation. However, if the hypothesis, stated in the second paragraph of this paper, is correct, factors other than this may be responsible.

In the skin temperatures there was an initial fall, followed by a rise, but

the changes were statistically insignificant; the temperature of the forehead was, however, significantly changed.

ACETYLCHOLINE. Ten experiments were done using freshly prepared acetylcholine in a dosage of 200 mgm. subcutaneously.

Temperature. The maximal average increase in temperature occurred in the stomach, where, after two hours, the mean rise was 0.16°C.; at the same time the mean rise in rectal temperature was 0.08°C. The mean recorded temperature of the mouth fell and rose by insignificant amounts.

The sharp rise of the intragastric temperature at the two-hour reading is interesting as it is comparable to the late changes in blood pressure and an increase of gastric acids which were observed. Since the change in temperature at the two-hour reading is the only rise which is statistically significant, this response in the intragastric temperature seems important, if we admit a possible influence of vasoconstriction and vasodilatation on the temperature. One is inclined to attribute the lateness of the effect to slow absorption of the drug rather than to a latent period of reaction.

Unfortunately cutaneous temperatures were not observed in this group of experiments. Kennedy and Barker found a marked rise in the temperatures of the digits after the subcutaneous administration of acetylcholine in 44.8 per cent of the number of digits studied, irrespective of the condition of health of the subject. If any dissociation of peripheral and visceral reaction takes place after acetylcholine, it is not easily apparent from these data.

ALCOHOL. Ten experiments were carried out by giving to the subjects orally 5 cc. of 95 per cent alcohol in 45 cc. tap water which was heated to the temperature of the fasting stomach.

Temperature. The changes in temperature with alcohol showed a greater variation than with the other drugs. The mean rise in rectal temperature over the whole period of observation was 0.18°C. The oral temperature rose sharply the first five minutes, a mean rise of 0.11°C., and continued to rise to a mean increase of 0.23°C. The quick initial increase in oral temperature may have been due in part to local vasodilatation, in part to the chemical reaction and in part to the temperature of the alcohol which was heated to the temperature of the fasting stomach. The rise of oral and rectal temperature was not simultaneous with that of the stomach.

Following ingestion of the alcohol there was the usual feeling of warmth in the epigastrium. That this was accompanied by an actual increase in intragastric temperature is seen in the fact that the intragastric temperature, after an initial mean decline of 0.175°C., rose sharply in thirty minutes an average of 0.41°C., and then dropped gradually to about fasting value for the remaining time of the experiments. The initial fall was in great part most likely caused by the temperature of the alcohol meal not

being exactly that of the stomach. The subsequent rise may possibly have been due to vasodilatation, but if vasodilatation is a factor, it is a negligible one. It was believed that the hygroscopic chemical reaction of the alcohol would generate sufficient heat to cause this definite rise.

This hypothesis was substantiated by the fact that a mixture in vitro of 5 cc. of hydrochloric acid and 5 cc. of 95 per cent alcohol, and that a mixture, in similar proportions, of distilled water and alcohol also increased in temperature to a comparable degree.

ARTIFICIALLY INDUCED RISES IN TEMPERATURE. Readings were also taken on subjects who received "T.A.B." vaccine. Results from five experiments were chosen because of completeness. Four of the patients studied had thrombo-angiitis obliterans and one had multiple sclerosis. The dose of vaccine varied from 25,000,000 to 200,000,000 bacteria. Three experiments were carried over a period of four hours, one over three hours, and one over two hours. The shorter experiments were terminated early because of severe pain in the extremities. All of the subjects had mild chills. The thermocouple was inserted when the subject felt the beginning of the reaction, so that the total rise of temperature was probably not obtained in all cases. Rectal, oral, and dermal temperatures and blood pressure readings were recorded.

In this group of experiments the blood pressure fell slightly, a drop of 4 mm. of mercury systolic and 6 mm. diastolic. The mouth temperature rose an average of 0.56°C., the rectal an average of 0.59°C. and the intragastric an average of 1.0°C. The total mean increase in the dermal temperature was 7.0°C. Thus, a fairly close parallelism in the increases of the mean temperatures is seen. It seems reasonable to believe that if the general body temperature should rise, the intragastric temperature should also increase, if there is no local condition to influence it otherwise. Perhaps if these observations had been extended over a longer period, greater variations in temperature might have been noted.

CONTROL. The control group of experiments consisted of identical procedures under identical conditions on ten subjects with similar diagnoses, but no drug (or test meal) was administered. The results were as expected except for the intragastric temperature, which showed a maximal average decline of 0.18°C. in the period of two hours. The rectal temperatures made a gradual rise as in the other groups. The mouth temperatures rose initially and then fell to about the fasting value after the first thirty minutes.

What the drop in intragastric temperature means is difficult to say. If the average readings are true indications, then the intragastric temperature can change independently of other parts of the body. It seems improbable that an organ situated in the center of the body and so closely connected with it by vessels and nerves can lower its temperatures while the body

temperature as measured by mouth and rectum rises, unless there is dissociation of splanchnic and peripheral temperature regulating mechanisms. A viscero-vasomotor action of some nature may account for the phenomenon. The cutaneous temperatures of the same group of subjects revealed a fall-rise sequence in three, a rise in six, and no change in one. It may be significant that while in half the experiments the intragastric temperature fell, in a similar proportion the cutaneous temperature rose.

The same dissociation of visceral and peripheral reactions was seen in the individual control experiments. On the face of it, one would be inclined to dismiss all the other apparent dissociations following the use of drugs, because in the control experiments it occurred with no stimulation. It is to be remembered that the gastric acids had been stimulated by the presence of the tube, and this also may have been sufficient to incite the

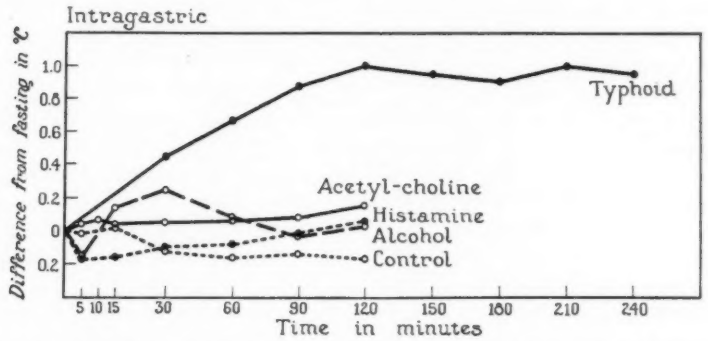

Fig. 1. Intragastric temperatures after various stimuli, and under controlled conditions with no stimuli.

autonomic dissociation to the point of causing the same results as after chemical stimulation.

COMMENT. The variations recorded in these data, even though small are probably true. On a statistical basis, the changes in intragastric temperature (fig. 1) are significant after administration of typhoid vaccine, probably significant in the control group, and not significant after administration of histamine, of alcohol and of acetylcholine. The rectal temperatures were changed significantly after administration of acetylcholine, of histamine, and of alcohol, and were questionably changed in the two other groups. The oral temperature showed significant changes after administration of histamine and of alcohol, and in the control group, probably significant changes after administration of typhoid vaccine, and no significant changes after administration of acetylcholine. The changes in dermal temperature were significant after administration of typhoid vac-

cine, probably significant after administration of alcohol, and in the control group, and not significant after administration of histamine, although the temperatures of the forehead after administration of histamine were significantly varied. It is true that the individual changes in temperature

TABLE 1

Mean differences from fasting temperatures

	TIME	STOMACH	RECTAL	MOUTH	SKIN	FOREHEAD
		Mean ± probable error	Mean ± probable error	Mean ± probable error	Mean ± probable error	Mean ± probable error
	minutes					
Controls, 10 experiments	5	−0.018 ± 0.013	+0.005 ± 0.003	+0.172 ± 0.025	+0.022 ± 0.229	
	15	+0.005 ± 0.025	+0.016 ± 0.011	+0.180 ± 0.025	+0.58 ± 0.32	
	30	−0.130 ± 0.040	+0.056 ± 0.016	+0.210 ± 0.028	+1.22 ± 0.287	
	60	−0.175 ± 0.043	−0.050 ± 0.014	+0.161 ± 0.009	+1.82 ± 0.349	
	90	−0.150 ± 0.052	+0.065 ± 0.020	−0.072 ± 0.014	+2.14 ± 0.580	
	120	−0.185 ± 0.048	+0.093 ± 0.015	+0.103 ± 0.020	+1.31 ± 0.836	
Acetylcholine, 10 experiments	5	+0.045 ± 0.031	+0.034 ± 0.012	−0.006 ± 0.003		
	10	+0.070 ± 0.036	+0.044 ± 0.008	+0.008 ± 0.010		
	15	+0.040 ± 0.030	+0.039 ± 0.010	+0.008 ± 0.010		
	30	+0.050 ± 0.034	+0.044 ± 0.008	+0.048 ± 0.023		
	60	+0.065 ± 0.047	+0.055 ± 0.010	−0.057 ± 0.062		
	90	+0.085 ± 0.047	+0.060 ± 0.012	−0.063 ± 0.061		
	120	+0.155 ± 0.047	+0.076 ± 0.005	+0.031 ± 0.037		
Typhoid, 5 experiments	30	+0.44 ± 0.12	+0.09 ± 0.071	+0.22 ± 0.06	+4.82 ± 0.88	
	60	+0.67 ± 0.15	+0.39 ± 0.040	+0.36 ± 0.10	+5.58 ± 0.62	
	90	+0.88 ± 0.16	+0.42 ± 0.130	+0.42 ± 0.12	+5.52 ± 0.53	
	120	+1.01 ± 0.23	+0.56 ± 0.202	+0.51 ± 0.178	+6.20 ± 0.89	
	150	+0.96 ± 0.286	+0.51 ± 0.276	+0.49 ± 0.266	+6.82 ± 0.566	
	180	+0.91 ± 0.317	+0.49 ± 0.276	+0.40 ± 0.232	+6.85 ± 0.384	
	210	+1.00 ± 0.366	+0.50 ± 0.366	+0.42 ± 0.315	+6.37 ± 0.561	
	240	+0.95 ± 0.596	+0.59 ± 0.556	+0.56 ± 0.457	+7.00 ± 0.476	
Alcohol, 10 experiments	5	−0.175 ± 0.060	+0.050 ± 0.015	+0.111 ± 0.017	+0.115 ± 0.128	
	15	+0.145 ± 0.074	+0.076 ± 0.015	+0.143 ± 0.022	+0.770 ± 0.267	
	30	+0.245 ± 0.086	+0.127 ± 0.028	+0.199 ± 0.031	+0.990 ± 0.441	
	60	+0.085 ± 0.025	+0.160 ± 0.029	+0.216 ± 0.030	+1.59 ± 0.322	
	90	−0.025 ± 0.044	+0.171 ± 0.032	+0.221 ± 0.030	+1.185 ± 0.344	
	120	+0.011 ± 0.024	+0.183 ± 0.033	+0.226 ± 0.033	+0.072 ± 0.428	
Histamine, 13 experiments	5	−0.188 ± 0.141	+0.052 ± 0.010	+0.071 ± 0.017	−0.146 ± 0.162	+1.49 ± 0.282
	15	−0.169 ± 0.136	+0.071 ± 0.021	+0.087 ± 0.022	−0.046 ± 0.250	+1.83 ± 0.223
	30	−0.163 ± 0.135	+0.102 ± 0.022	+0.133 ± 0.041	+0.311 ± 0.406	+1.92 ± 0.174
	60	−0.092 ± 0.048	+0.153 ± 0.025	+0.234 ± 0.029	+1.13 ± 0.517	+1.53 ± 0.207
	90	+0.019 ± 0.034	+0.175 ± 0.028	+0.253 ± 0.027	+0.525 ± 0.569	+1.19 ± 0.225
	120	+0.050 ± 0.044	+0.191 ± 0.031	+0.242 ± 0.029	+0.423 ± 0.570	+1.30 ± 0.279

in most of the cases were slight enough to be within the limits of instrumental error. Furthermore, the varying approximation of the gastric walls, over the thermocouple, may be a factor. However, despite the fact that some of these changes are statistically not significant, the smoothness of the curves, with their consistent rises over a period of two hours, leads

one to believe that these changes are actual, more so because the changes in stomach, mouth and rectum are rather closely parallel. The concept of a dissociation of splanchnic and peripheral mechanisms and the support brought to this concept by some of these experiments, suggests interesting physiologic problems.

It was at first thought that the steady rise of the body temperature at all three points might be diurnal. The one marked discrepancy was the fall in the intragastric temperatures of the control group. If this latter is a true observation, then we must explain the other findings on another basis.

There is no absolute reason why vasomotor factors might not be responsible in some degree. It is certain that vasodilating drugs have a visceral effect, but perhaps not a consequent effect on local temperature in the viscera. Rich noted a local vasodilator effect of histamine on the omentum of the etherized cat. Florey and Carleton, observing under saline the mesenteric capillaries of the anesthetized cat, found that injection of histamine in the saphenous vein produced dilatation of capillaries and opening up of many which had not been apparent previously. However, if dilatation of the intrinsic gastric vessels occurs, and a rise in temperature obtains, the rise would be so small and so transient, due to the rapidity of the gastric circulation, that its measurement would be difficult. An increase in local heat was certainly obtained and probably to a greater degree than was actually noted. That this increase in temperature was due to an increase in local blood volume is questioned, however.

A possible explanation may be found in an increase in local metabolism. The highest rise in intragastric temperature, exclusive of typhoid shock, was noted after histamine, which, as might be expected, caused also the greatest increase in gastric secretory activity. It is generally accepted that histamine acts directly on the gastric cells, as shown by its action on a denervated stomach and isolated gastric pouches. It is possible, therefore, that the effects noted in these experiments are the result of action primarily on the skin, secondarily on the gastric vessels and stomach motility, and superimposed on the direct stimulation of the intrinsic cellular activity of the stomach. Müller and Kast, explaining the effect of heat and cold on the splanchnic organs, wrote of the splanchnoperipheral balance; that is, the hotter the skin, the less internal activity occurs on account of vasoconstriction. There may be visualized a process such as this: increasing peripheral vasodilatation → decreasing gastric vasodilatation or actual constriction → decreased blood supply to the stomach → decreased cellular metabolism → decreased local temperature. But this took place only in the control group where no vasodilatation was induced. On the other hand, following the primary peripheral vasodilatation, vasoconstriction may occur in the cutaneous vessels, causing compensatory vasodilatation of the visceral vessels with increased blood supply to the stomach, increased

cellular metabolism and increased local temperature. The first of the phases may be merely transient, as suggested by Petersen and Müller. This would explain the initial fall and subsequent slow rise of intragastric temperature following the administration of histamine.

Recently some French authors suggested the use of vasodilating drugs in the treatment of peptic ulcer on the basis that a visceral vasodilatation would assist in the healing process. No evidence can be adduced from these experiments to support this contention.

The interpretation of the importance of these changes is difficult. They are certainly not of a degree to be of immediate therapeutic interest. The changes noted are slight; but in view of the fact that they occur in the center of the body, they probably are significant.

Observations were made coincidentally with the studies of temperature on the blood pressure and the aspirated gastric content. These are somewhat irrelevant to the main object of this study, and for that reason detailed reference to them is omitted. Following administration of histamine the blood pressure fell an average of 6 mm. of mercury systolic and 10 mm. diastolic. The free acids, total acids, and total chlorides formed the usual curve observed in response to histamine. After administration of acetylcholine the blood pressure dropped 9 mm. of mercury systolic and 4 mm. diastolic. The acids and chlorides made a sharp rise and a sharp fall, and then a steady rise for the remainder of the period of observation. After administration of alcohol the blood pressure varied only slightly, while the curves representing the change in the acids and chlorides were similar to those representing the same constituents after administration of histamine, but were flatter. In the studies of artificially induced fever the blood pressure dropped an average of 4 mm. of mercury systolic and 6 mm. diastolic. In the control group the acids and chloride rose and fell, due, presumably, to the stimulating effect of the tube in the stomach. There was no change in the readings of blood pressure.

SUMMARY AND CONCLUSIONS

1. Studies have been made concerning the variations of intragastric temperatures in subjects suffering with ulcers in the gastro-intestinal tract in relation to vasodilating drugs and test meals.

2. After stimulation with histamine, the mean rise in oral temperature was 0.25°C., and the mean rise in rectal temperature was 0.19°C. The mean fall in intragastric temperature was 0.18°C. initially, and then it made a rise above the initial temperature comparable to the rise noted in rectal and oral readings.

3. After acetylcholine the intragastric temperature rose on an average of 0.16°C. as compared to a rise of 0.08°C. for the rectal temperatures and insignificant variations in oral temperatures; it made a sharp rise at the

two-hour reading. A peripheral and visceral dissociation was not so marked after the administration of acetylcholine as when histamine was used.

4. After the administration of alcohol the rectal and oral temperatures rose gradually. After an initial drop, the mean rise of intragastric temperature was 0.4°C.; then the temperature dropped to normal. A definite splanchnoperipheral dissociation was apparent in 60 per cent of the experiments.

5. The dissociation of visceral from peripheral reactions affecting the temperature was most apparent after the administration of histamine, less so but fairly definite after alcohol and variable after acetylcholine.

6. In the presence of fever artificially produced the maximal mean rise of oral temperature was 0.56°C., of the rectal temperature 0.59°C., and of the intragastric temperature, 1.0°C. In only 20 per cent of the cases was a definite splanchnoperipheral dissociation seen, although this phenomenon may have been overloooked in the other 80 per cent.

7. The theoretic relationship of splanchnic vasodilatation to temperature has been considered.

8. Additional evidence has been presented to support the concept of a splanchnoperipheral balance.

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THE SUGAR IN BLOOD AND SUBCUTANEOUS LYMPH FOLLOWING INSULIN ADMINISTRATION

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The object of this work is to investigate changes in extravascular sugar following insulin administration in normal dogs and in a diabetic dog, and to compare these values with simultaneous observations of the blood-sugar levels.

Subcutaneous lymph was chosen as a medium in which to examine these changes. Arnold and Mendel (1927) have shown that the sugar content of thoracic-duct lymph falls after insulin administration. Thoracic-duct lymph is, however, a mixture of fluids which originate in the main from the highly permeable capillaries of the liver and intestines and cannot be associated with any definite tissue area. Furthermore, the analyses reported were made on pooled lymph samples obtained from an amytalized dog without effort to synchronize the taking of blood and lymph specimens.

In our experiments foot lymph was collected from unanesthetized dogs, a lymphatic having been cannulated under novocaine (White, Field, and Drinker, 1933). With the foot kept in gentle motion by walking the dog about the laboratory, subcutaneous lymph flowed freely from the cannula. Under these conditions we feel that the composition of lymph closely resembles that of the fluid environment of the tissues in the area drained by the lymphatics (Drinker and Field, 1933).

EXPERIMENTAL. *Sampling.* Lymph was obtained from a cannula inserted in a lymphatic trunk in the ankle. A small amount of dry heparin placed in the cannula prevented coagulation. Under these conditions and with the dog walking quietly about the laboratory a continuous flow of lymph was assured, and 0.1 cc. samples were taken by means of a Folin micro-sugar pipette. Blood was withdrawn by means of a syringe from a femoral artery. It was transferred to a centrifuge tube containing sufficient oxalate to prevent coagulation, centrifuged immediately, and 0.1 cc. of plasma was taken for analysis. Normal samples of plasma and lymph were taken in duplicate or triplicate and were followed by a subcutaneous injection of insulin (Eli Lilly & Company).

Analysis. Sugar analysis by the Folin micro-ferricyanide method was begun immediately after sampling. Lymph and plasma proteins were determined refractometrically.

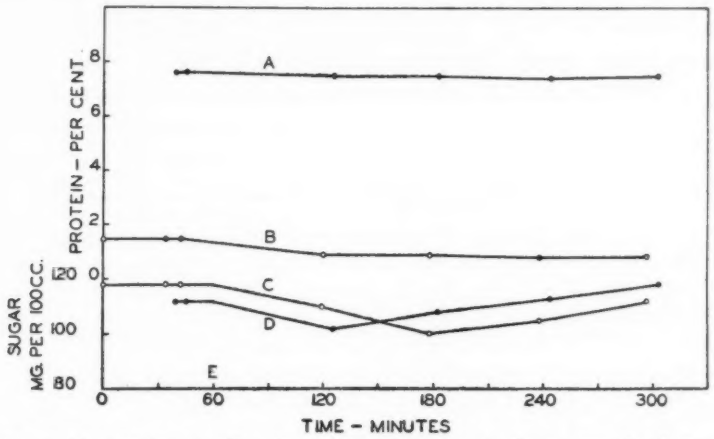

Fig. 1. Normal dog 16. Female, weight 22.5 kgm. *A*, plasma protein; *B*, lymph protein; *C*, lymph sugar; *D*, plasma sugar; *E*, 0.1 U insulin per kilo administered.

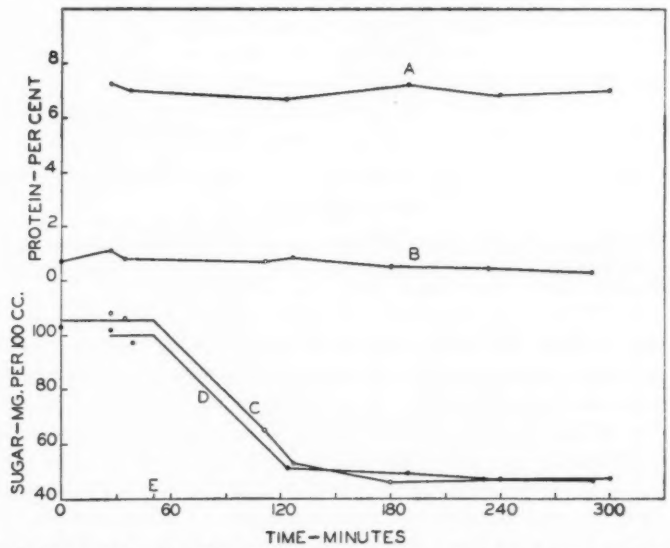

Fig. 2. Normal dog 16. Female, weight 22.5 kgm. *A*, plasma protein; *B*, lymph protein; *C*, lymph sugar; *D*, plasma sugar; *E*, 1.0 U insulin per kilo administered.

DISCUSSION OF RESULTS. Figure 1 shows the changes in lymph and plasma sugar of a normal dog following a small dose of insulin (0.1 U per kilo). The sugar values are based on the water contents of the two fluids. The effect of this injection is a small and transient lowering of the plasma and lymph-sugar levels. A definite time lag of the lymph-sugar concentration behind that of the plasma will be noted throughout.

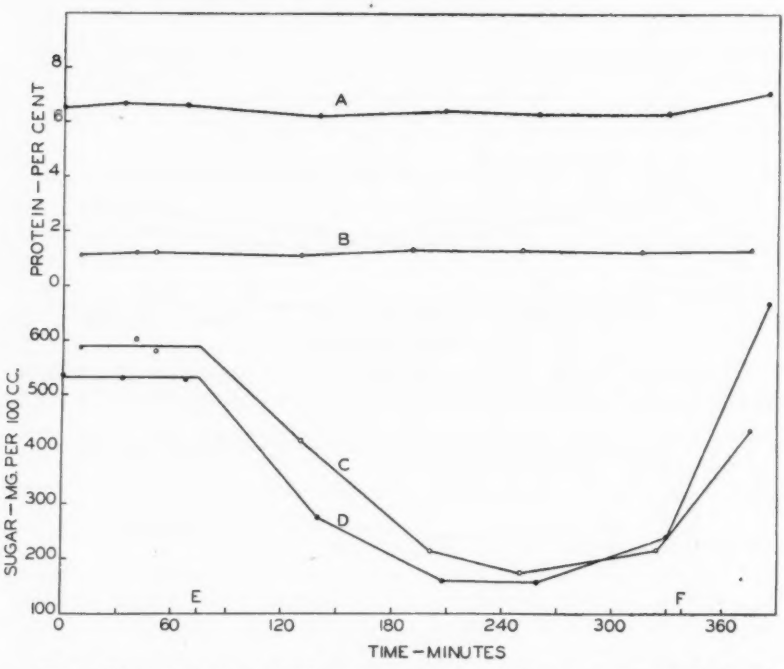

Fig. 3. Diabetic dog 149. Male, weight 10.2 kgm. *A*, plasma protein; *B*, lymph protein; *C*, lymph sugar; *D*, plasma sugar; *E*, 0.1 U insulin per kilo administered; *F*, dog fed.

Figure 2 shows the result of a larger dose of insulin (1.0 U per kilo) administered to the same dog. In this case a marked and lasting reduction of the plasma sugar occurred. As in the previous experiment, during the period of change the lymph-sugar level follows that of the plasma, falling at approximately the same rate and lagging by a definite time interval. At the end of about one hour the plasma sugar has stopped changing, and from here on remains approximately constant. A little later the lymph sugar comes into equilibrium with that of the plasma and remains approximately so throughout the course of the experiment. The results of a simi-

lar experiment carried out on a diabetic dog are shown in figure 3. This animal was depancreatized 112 days previously and was upon a diet of 800 grams of lean meat, 130 grams of glucose, and 10 grams of Vitavose supplemented by 20 units of insulin injected daily. It had fasted and had been without insulin for 18 hours prior to the beginning of the experiment. Examination of figure 3 shows that plasma sugar and lymph sugar maintain a relationship similar to that in the normal animal. Just as in the normal dog, the lymph-sugar level exceeds the arterial plasma-sugar level at the start of the experiment, and this relationship tends to be maintained throughout the period of insulin effect. This finding is not invariable in normal dogs, the quantities of sugar in plasma and lymph being frequently practically equal. A lymph sugar distinctly lower than the arterial plasma sugar has never been found. We can offer no explanation for this state of affairs. It may be pointed out that the changing amounts of sugar in plasma and lymph occur with lymph and plasma proteins at very constant levels and that the maintenance of the plasma sugar-lymph sugar relationship after the injection of insulin indicates that the rate of diffusion of sugar from blood to lymph is extremely rapid. In this respect there appears to be no difference between the normal and the diabetic animal.

SUMMARY

1. Subcutaneous lymph and arterial blood have been collected from normal dogs and from a depancreatized dog under local anesthesia.

2. Lymph sugar—extravascular sugar—is higher or equals the sugar in arterial plasma. It is never significantly lower.

3. The injection of insulin causes a fall in plasma and lymph sugar, the values remaining very close together.

4. The diffusion of sugar from blood to lymph is similar in the normal and the diabetic dog.

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THE RELATION OF VITAMIN B REQUIREMENT TO METABOLISM

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In a recent paper Cowgill (1932) presented data on the relation of vitamin B requirement to live weight for the pigeon, dog, rat, and mouse. For any one species of animal the vitamin B requirement was found to be proportional to the 5/3 power of the live weight of the animal, viz.:[1]

$$V = k_s W^{1.66} \tag{1}$$

in which the factor of proportionality, k_s, is constant for the species. The values of the constant, k_s, for the various species were found to be inversely proportional to the respective maximum normal live weights for the species:

$$\log k_s = \log k - \log \overline{W} \tag{2}$$

in which the constant, k, is constant for all species and $\overline{W}$ represents the maximum normal live weight for any one species. From equations (1) and (2) the following relation was obtained:

$$V = kW^{0.66}\frac{W}{\overline{W}} \tag{3}$$

On the basis of these results it was concluded that the vitamin B requirement of an animal is proportional to the metabolism, multiplied by a factor correcting for age.

These results were of particular interest to the author of the present paper because, as will be shown later, they bear a marked similarity to some results obtained in attempting to estimate the maintenance requirements of growing chickens.

Hendricks, Jull, and Titus (1931) have suggested a physiological interpretation of the equation of the curve of diminishing increment, an equation first used by Spillman (1924) for describing the relation between the live

[1] The notation employed in the present paper is slightly different from that found in Cowgill's paper.

weight and feed consumption of a growing animal. The differential form of this equation may be written:

$$\frac{dW}{dF} = C - mW \tag{4}$$

from which the following time relationship may be readily obtained:

$$\frac{dW}{dt} = C\frac{dF}{dt} - mW\frac{dF}{dt} \tag{5}$$

Equation (5) shows that the gain in live weight, per unit time, is the algebraic sum of a positive increment, proportional to the amount of feed ingested, per unit time, and a negative increment, proportional to the product of the live weight and the amount of feed ingested, per unit time. One is led to the natural conclusion that the constant, C, represents the gain which the animal is capable of making for each unit of feed consumed if no feed were required for other metabolic processes, and that the expression, $mW\frac{dF}{dt}$, represents the loss in live weight, per unit time, due to metabolic processes other than growth for which some of the feed consumed must supply the necessary energy. The expression, $mW\frac{dF}{dt}$, may be regarded as representing the maintenance requirement of the animal, expressed in terms of an equivalent amount of body tissue rather than in terms of a quantity of feed or in thermal units.

There appears to be a general impression to the effect that the maintenance requirement of an animal should be represented mathematically as being proportional to a power of the live weight. The author has no desire whatever to defend this point of view. The purpose of the present paper is merely to present some results obtained when an expression of the form, aW^n, is substituted for $mW\frac{dF}{dt}$ in equation (5). The equation then becomes:

$$\frac{dW}{dt} = C\frac{dF}{dt} - aW^n \tag{6}$$

Hendricks, Jull, and Titus (1932) have discussed data, obtained at the U. S. Animal Husbandry Experiment Farm, Beltsville, Md., on the growth of fourteen lots of cross-bred chickens (approximately 30 per lot) which were reared on different levels of nutrition. Only a brief summary of the experimental conditions need by given here. Seven of the lots consisted of cockerels and seven of pullets, the sexes being separated at hatching time. The seven lots of each sex were fed on levels of nutrition ranging

from ad libitum feeding to extremely subnormal levels. The birds were weighed once each week until they attained the age of one year, except for a few lots which were discontinued before that time. The observed feed consumption was corrected for egg production for those lots of pullets in which eggs were produced.

In the study herein reported, the constants, C, a, and n, in equation (6) were evaluated for each lot of birds. The equation is not integrable. However, since the birds were weighed each week and feed consumption records were available for the same intervals, the values of the constants could be approximated quite well by substituting finite increments for the infinitesimals and fitting the resulting equation:

$$\frac{\Delta W}{\Delta t} = C\frac{\Delta F}{\Delta t} - aW^n \tag{7}$$

to the data by the method of least squares. The values of W used in fitting this equation were the average values for each weekly interval, calculated

TABLE 1

Values of C, a, and n calculated for each lot of cockerels

LOT	LEVEL OF NUTRITION	C	a	n
	per cent of ad lib. consumption			
1	100.0	0.3025	0.0029226	1.398
2	65.7	0.3113	0.0005883	1.595
3	56.4	0.3303	0.0016712	1.472
4	47.0	0.2973	0.0005789	1.601
5	37.6	0.3231	0.0197662	1.176
6	28.2	0.4636	0.0885969	1.051
7	18.8	0.4091	0.0806675	1.051

by taking one-half the sum of the live weight at the beginning and at the end of the interval.

The results of fitting equation (7) to the data obtained for the cockerels are given in table 1. All weights were expressed in grams.

During the course of the computations it became evident that equation (7) would fit the data very well for a wide range of arbitrary values which might be assigned to the constant, n. In particular, it was found that when the value, 5/3, was arbitrarily assigned to n and the remaining two constants were evaluated by the method of least squares, the standard error of estimate was actually less than that obtained when n was also evaluated by the method of least squares. In other words, the improvement in the fit of the equation when the most probable value of n was used was more than nullified by the loss of the additional degree of freedom.

Therefore, it did not seem worth while to follow the laborious procedure of fitting the general form of equation (7) to the data for the pullets.

Tables 2 and 3 present the results of fitting equation (7) to the data for the cockerels and the pullets when n was assigned the value, 5/3. The maximum live weight attainable by each lot of birds on the given level of nutrition has been included in these tables as a matter of interest. The values given are those reported by Hendricks, Jull, and Titus (1932).

TABLE 2

Values of C and a calculated for each lot of cockerels when the value, 5/3 was assigned to n

LOT	LEVEL OF NUTRITION	MAXIMUM LIVE WEIGHT	C	a
	per cent of ad lib. consumption	*grams*		
1	100.0	3637	0.2751	0.0002968
2	65.7	2956	0.3038	0.0003253
3	56.4	2672	0.3076	0.0003406
4	47.0	2275	0.2908	0.0003427
5	37.6	1725	0.2535	0.0003692
6	28.2	972	0.3063	0.0008540
7	18.8	639	0.2750	0.0010367

TABLE 3

Values of C and a calculated for each lot of pullets when the value, 5/3 was assigned to n

LOT	LEVEL OF NUTRITION	MAXIMUM LIVE WEIGHT	C	a
	per cent of ad lib. consumption	*grams*		
8	100.0	2055	0.3334	0.0006435
9	87.7	2287	0.3419	0.0005457
10	76.3	2153	0.3500	0.0005506
11	64.5	1993	0.2976	0.0004249
12	52.4	1653	0.3023	0.0004884
13	39.3	1320	0.2611	0.0004570
14	26.2	681	0.2461	0.0008506

The values of the constant, a, are related to the maximum live weights in a manner perfectly analogous to the relation between the constant, k_s, and the maximum normal live weight for the species given by equation (2). The logarithms of the values of a have been plotted against the logarithms of the maximum live weights in figure 1. The solid line in the figure is the straight regression line fitted by the method of least squares. The broken line is the best fitting line which could be obtained when the slope was arbitrarily assigned the value, -1.00000. The equations of these regression lines are respectively:

$$\log a = -0.65583 \log \overline{W} - 1.17981 \tag{8}$$

$$\log a = \quad - \log \overline{W} - 0.06563 \tag{9}$$

Considering the variability of the data, there is no reason to believe that the differences between these two equations are significant. As a matter of fact, there is a logical reason for expecting the slope of the regression line to be equal to -1.00000.

It may be shown that the value of the constant, m, in equations (4) and (5) is inversely proportional to the maximum live weight attainable on a given level of nutrition, provided the constant, C, has the same value regardless of the level of nutrition on which the birds were reared. An examination of the values of C in tables 2 and 3 shows that these values

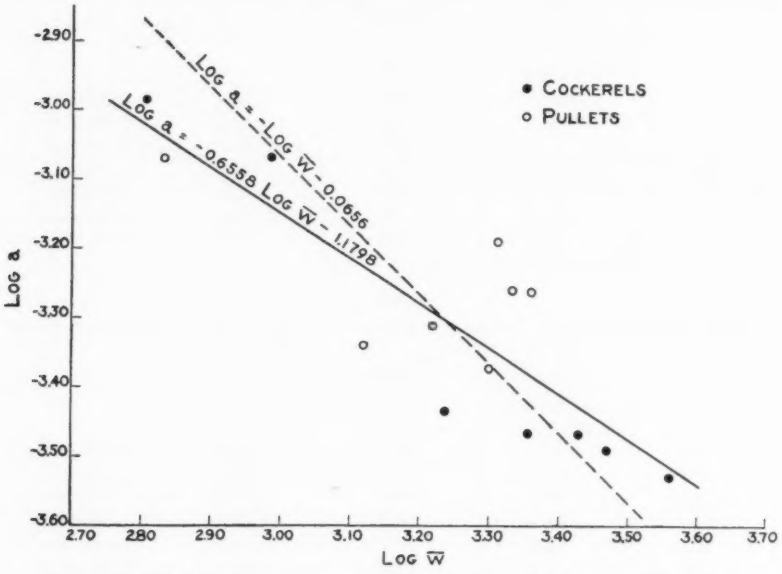

Fig. 1. Relation of the constant, a, to the maximum live weight attainable

appear to be uncorrelated with the respective levels of nutrition. Furthermore, if equations (5) and (6) are capable of fitting the same set of data, it seems reasonable to suppose that the expression, $mW\frac{dF}{dt}$, in equation (5) is very nearly equivalent to the expression, aW^n, in equation (6) since the other corresponding terms in the two equations are identical. It follows that the constant, m, must be at least approximately proportional to the constant, a, and if the former is inversely proportional to the maximum live weight, the latter must also be inversely proportional to the maximum live weight.

These results may be compared with Cowgill's findings relating to the

vitamin B requirement if the maximum live weights attainable by animals of the same species, reared on different levels of nutrition, can be regarded as being comparable to the maximum normal live weights of animals of different species. This point of view appears entirely justifiable to the author of the present paper.

The results of the study herein reported, then, indicate that the vitamin B requirement of an animal is proportional to the metabolism, rather than to the product of the metabolism and a factor correcting for age as suggested by Cowgill. The metabolism, at least in the case of the chicken, appears to be proportional to the 5/3 power of the live weight.

SUMMARY

In an attempt to estimate the maintenance requirements of growing chickens, some results were obtained which suggest that the metabolism of an animal is proportional to the 5/3 power of the live weight.

Since recently published work by Cowgill (1932) shows that the vitamin B requirement of an animal is proportional to the 5/3 power of the live weight, it is probable that the vitamin B requirement is proportional to the metabolism.

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FURTHER QUANTITATIVE STUDIES IN ABSORPTION

The Influence of Acetylcholin on the Absorption of Glucose

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In a preceding paper (1933) it was shown that various hormones, including adrenalin, thyroxin and insulin, showed a specific influence on the permeability of the gut under the conditions of a constant perfusion rate of Ringer's solution through the blood vessels supplying the gut. It seemed desirable to include acetylcholin among the substances studied. The effect of acetylcholin on absorption was of interest because this substance, similar to those investigated in a previous paper, seems to have some hormone characteristics (Le Heux) and displays these hormonal effects in the gut. Furthermore acetylcholin and adrenalin frequently show antagonistic effects and the problem arises whether or not these two substances influence permeability in opposing directions.

The method is the same as described in the first number of this series. Only one slight modification was introduced, the ligation of the spleen. It was found that not infrequently traces of blood appeared in the perfusion fluid even after 40 minutes of perfusion with Ringer's solution. When the spleen is regularly ligated no traces of blood are observed.

Thirty experiments were performed with acetylcholin in concentrations of from 1:50,000 to 1:40,000,000. The solutions were made up for each experiment. These concentrations all produced a heightened activity of the gut musculature, usually of the nature of spastic contractions. We could observe no regular effect on the perfusion pressure necessary to maintain a constant rate, which is a measure in this preparation, of the diameter of the blood vessels. If any dilatation occurred, it was masked by the increased activity of the musculature, which mechanically affects the perfusion rate.

The following protocol shows a typical result with a strong acetylcholin concentration.

Absorption experiment. (Rana Esculenta) 12/27/32. Perfusion of blood vessels with 1, Ringer solution, and 2, Ringer + acetylcholin

[1] Aided by a grant from the Ella Sachs Plotz Foundation.

1:50,000. Gut is perfused with 3.15 per cent glucose solution. Sample of perfusion fluid taken in 10 minute intervals.

TIME	NUMBER	PERFUSION FLUID	AMOUNT OF FLUID	CONCENTRATION OF GLUCOSE
			cc.	*mgm. per cent*
3:20–3:30	1	I	6.2	3.1
3:30–3:40	2	I	6.2	3.2
3:40–3:50	3	II	5.7	2.7
3:50–4:00	4	II	6.2	1.1
4:00–4:10	5	I	6.3	1.3
4:10–4:20	6	I	5.6	1.7
4:20–4:30	7	II	5.8	0.9
4:30–4:40	8	II	6.0	0.3

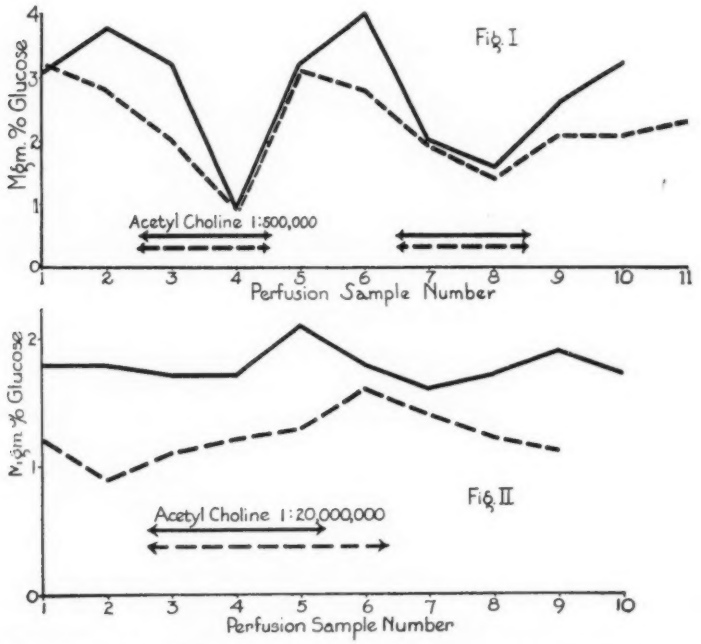

Fig. 1. Perfusion of the blood vessels supplying the gut with Ringer and Ringer + acetylcholin 1:500,000 respectively. Perfusion of the gut with 3.15 per cent glucose. Ordinate: Concentration of glucose in mgm. per cent in the perfusion fluid which leaves the portal vein. Abscissa: Perfusion sample number (samples taken in 10 min. intervals).

Fig. 2. As in figure 1 but acetylcholin 1:20,000,000.

It is evident that the amount of glucose which permeates through the gut decreases under the influence of acetylcholin. This decrease is partially reversible, as is indicated in the increase in concentration during periods 5 and 6. It is again followed by a strong decrease in concentration due to the second application of acetylcholin (periods 7 and 8). The amount of the fluid which passes through the blood vessels of the gut varies less than 10 per cent, so that these variations certainly can not account for the remarkable alterations in glucose concentration. The conclusion seems to be inevitable that in high concentrations acetylcholin decreases the permeability of the gut. Some curves obtained from experiments with acetylcholin 1:500,000 illustrate these results (fig. 1). In 17 experiments performed with acetylcholin varying from 1:50,000 to 1:5,000,000 an increase in permeability to sugar never occurred. In five experiments no effect was observed; but if a change in glucose concentration occurred, it invariably was a decrease.

Since in experiments with adrenalin a different effect was obtained with high and low concentration the question was investigated whether or not the influence of acetylcholin on permeability also depended on the concentration. The results were regular and showed that in low concentration (1:20,000,000) a reversible increase in glucose concentration occurred, indicating that the permeability of the gut was increased. The effects were slight but regular if acetylcholin was administered for a sufficient length of time (fig. 2). The lowest concentration in which acetylcholin caused a reversible increase in permeability to glucose was 1:40,000,000.

SUMMARY AND CONCLUSION

Acetylcholin increases reversibly the permeability of the gut to glucose in concentrations of 1:20,000,000 and 1:40,000,000. In high concentrations (1:50,000 to 1:2,000,000) it decreases reversibly the permeability of the gut to glucose. Since the effect of adrenalin on the permeability of the gut to glucose was exactly the opposite it may be said that acetylcholin and adrenalin have antagonistic effects in regard to permeability as they have in reference to many other autonomic functions.

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THE FUNDAMENTAL CHEMICAL CHANGES IN CONTRACTING MAMMALIAN MUSCLE

II. Changes in Lactic Acid, Phosphocreatine and Hexosephosphate in Stimulated Muscles of Rats

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In a previous paper (10) a new theory was proposed concerning the essential chemical changes involved in the performance of muscular work. The theory states that the normal energy-yielding reactions in muscle are oxidative rather than anaerobic; that anaerobic reactions are utilized only when the oxygen supply is inadequate. Further, the formation of hexosephosphate is not to be considered as an intermediate step in the production of lactic acid; rather is it one of the anaerobic reactions which yield energy—the formation of lactic acid from glycogen being the other major one. The function of phosphocreatine hydrolysis is that proposed by Fiske (2), i.e., the base liberated by this hydrolysis buffers the muscle against the lactic formed during oxygen deficiency. It is not to be considered as the primary source of the energy for contraction, as postulated by Lundsgaard (7).

The work referred to was done on the muscles of rabbits. In this species the resting muscles are not very vascular, the circulatory adjustment is slow, and large amounts of lactic acid and hexosephosphate are formed in a tetanus before the circulation adjusts itself. In view of Krogh's (6) findings that capillaries are more numerous in voluntary muscle of small animals than in large ones, it was thought advisable to repeat the observations on the rat, in which the possibility exists that the circulatory adjustment can be made more rapidly, with a resultant decrease in the demand for the anaerobic reactions.

Experimental. The general procedure was the same as that used previously: the animals were anesthetized with pentobarbital-sodium, 6.5 mgm. per 100 grams intraperitoneally, supplemented with ether. Both gastrocnemius muscles were dissected as free as possible without interfering with their blood or nerve supply. One muscle was then frozen in the resting state, *in situ*, with a mixture of powdered solid carbon dioxide and ether. The other was tetanized through the nerve for 5, 15 or 30 seconds,

TABLE 1

Changes in lactic acid, phosphocreatine and hexosephosphate contents of gastrocnemius muscles of rats after tetanus

Values expressed as milligrams per cent of P and of lactic acid

LACTIC ACID			PHOSPHORUS					
			Resting		Stimulated		Difference	
Resting	Stimulated	Increase	Inorganic	Inorganic plus phosphocreatine	Inorganic	Inorganic plus phosphocreatine	Inorganic = phosphocreatine hydrolysis	Hexosephosphate formation from phosphocreatine-P
A. Duration of tetanus 5 seconds								
18	43	25	22	84	30	74	8	10
24	35	11	17	78	34	73	17	5
28	56	28	16	85	22	78	6	7
16	43	27	16	81	23	72	7	9
22	35	13	13	85	21	76	8	9
16	49	33	15	78	27	69	12	9
21	58	37	17	78	28	72	11	6
13	62	49	16	89	32	78	16	11
16	61	45						
Average.....		30					10	8
B. Duration of tetanus 15 seconds								
15	41	26	18	90	28	75	10	15
22	64	42	15	95	30	81	15	14
22	78	56	17	72	34	61	17	11
15	79	64	16	76	39	63	23	13
26	76	50	20	79	32	70	12	9
14	89	75	16	87	35	65	19	22
12	107	95	22	76	36	63	14	13
Average.....		58					16	14
C. Duration of tetanus 30 seconds								
16	53	37	19	81	35	64	16	17
21	88	67	17	77	30	64	13	13
18	71	53	19	89	29	80	10	9
30	58	28	18	95	30	79	12	16
9	83	74	16	83	28	75	12	8
18	55	37	19	80	31	72	12	8
22	92	70	14	84	30	65	16	19
15	52	37	18	76	39	65	21	11
Average.....		50					14	13

Note: As explained in the text, the determinations of lactic acid and phosphorus were not done on the same animals.

and then frozen *in situ*; the stimulation was continued until the muscle was frozen. With these small muscles, weighing only a gram, freezing is instantaneous.

Trichloroacetic acid filtrates were prepared in the usual way, and determinations made of lactic acid, inorganic phosphate, and phosphate after acid hydrolysis at room temperature, which is the sum of inorganic and phosphocreatine phosphorus. As before, the difference in the hydrolysis values of the resting and stimulated muscles was taken as a measure of the amount of hexosephosphate formed. Adenosine triphosphate was not determined, as there is ample evidence that in such periods of tetanus the compound does not undergo any dephosphorylation (1), (10). Inorganic phosphate was precipitated by magnesia mixture.

Unfortunately, the amount of material furnished by one gastrocnemius is too small to permit of satisfactory analysis for both phosphorus and lactic acid. The use of muscle groups rather than single muscles is open to serious objection, and it was felt that nothing was to be gained by pooling samples. Hence lactic acid determinations were done on one series of animals, and phosphorus determinations on another; the average change in the one group is to be compared with the average change in the other. This procedure should permit correct interpretation if each series is sufficiently large.

Results. It can be seen from the table that for each duration of tetanus, there is a very wide range in the amount of lactic acid and hexosephosphate formed, and of phosphocreatine hydrolyzed. However, if we compare the *averages* for the groups of experiments, it is seen that *the lactic acid formed and phosphocreatine hydrolyzed are in nearly equimolecular ratio.* This is true for all three groups of experiments. It is also seen that there is more than half as much lactic acid and hexosephosphate formed, and more than half as much phosphocreatine hydrolyzed, in 5 seconds of tetanus as in 15 seconds. It is even more striking that the average lactic acid and hexosephosphate formed, and phosphocreatine hydrolyzed, are no greater in a 30 second tetanus than in one of half that duration. In other words, the muscle has maintained tension during the second half of a 30 second tetanus without any phosphocreatine hydrolysis or lactic acid formation.

Discussion. It is difficult to account for the wide variations within the groups on the basis either of Lundsgaard's hypothesis or the old Hill-Meyerhof theory. In a muscle frozen at the height of a short maximal tetanus there is little opportunity for phosphocreatine resynthesis or delayed lactic acid formation, as required by Lundsgaard's hypothesis. Hence there should not be such marked differences from one animal to another. Nor should there be such differences if the energy for contraction comes exclusively from lactic acid formation, as postulated by the

Hill-Meyerhof theory. However, on the basis that lactic acid production is an index of the oxygen deficiency, such results may be expected.

On this same basis it is easy to understand why the rates of lactic acid and hexosephosphate formation should decrease so rapidly as the tetanus is prolonged. The initial period of tension development and maintenance must perforce be largely anaerobic, for the oxygen tension in the resting muscle is very close to zero, as has been shown by Verzar (11). Therefore the accumulation of lactic acid and hexosephosphate must be greatest during this initial period, since whatever small amount of oxygen that may be present is rapidly used up and further oxidative reactions must wait upon the diffusion of oxygen into the tissues from the large number of capillaries which open up when the muscle is stimulated (6). It is obvious that this adjustment takes place more rapidly and more effectively in the rat than in the rabbit (10). In the former, lactic acid production becomes unnecessary in 15 seconds or less, while in the latter it is still furnishing a large part of the energy after 30 seconds' tetanus, and is in evidence even after one minute of continuous tetanus. Also, the amount of hexosephosphate formed is much greater in the rabbit than in the rat, for the same duration of tetanus.

The close parallel between the rates of formation of lactic acid and hexosephosphate that was observed in the rabbit is here noted again. This supports the view that the function of the formation of the two substances is the same, i.e., that both reactions serve to yield energy for the contraction process when the oxygen supply is inadequate.

The nearly equimolecular ratio between lactic acid formation and phosphocreatine hydrolysis is very strong evidence for the correctness of Fiske's interpretation of the rôle of phosphocreatine. He has shown that at pH 5.5 to 6.0, the hydrolysis of one mole of phosphocreatine will yield sufficient base to buffer against seven-eighths of a mole of lactic acid without any change in pH. According to the intravital staining experiments of Rous (9), the pH of the muscle fibers themselves is very close to 5.6. Fiske does not claim that the pH of the muscle fiber remains unaltered during activity, but from these data it is not necessary to assume any shift in pH. In addition to the large buffering effect of the phosphocreatine hydrolysis, additional buffer is liberated by the formation of hexosephosphate—which has pK_2' of 6.12, according to Irving and Fischer (5)—from phosphocreatine of pK_2' of 4.6 (2). From these values it can be seen that the amount of hexosephosphate formed is more than enough to furnish the remaining buffer substance necessary to keep the pH of the muscle fiber from decreasing.

In this connection it may be recalled that Meyerhof (8) has laid particular stress on the rôle of alkali protein in the neutralization of lactic acid in frog muscle. Rous' studies of the pH of the tissues shows that it is

highly improbable that such a mechanism exists in mammalian muscle, and the recent data of Hines and Knowlton (4) indicate that no base is available in rat muscle for combination with protein, as the total amount of potassium, hence of fixed base, is practically equivalent, stoichiometrically, to the total acid-soluble phosphorus. It thus becomes evident that in rat muscle alkali protein cannot serve for the neutralization of lactic acid.

A comparison of the present data with those previously obtained on rabbits shows a striking difference in the behavior of phosphocreatine in a 5 second tetanus. Whereas the rat muscle begins to hydrolyze phosphocreatine as soon as the lactic acid content is raised, there is no evidence of such hydrolysis in the rabbit in such a period of tetanus, even though the lactic acid increase is somewhat greater. It is extremely difficult to account for such a marked difference by Lundsgaard's hypothesis that the hydrolysis of phosphocreatine furnishes the energy for contraction. The buffer theory of Fiske, on the other hand, does furnish a satisfactory explanation, on the basis of the difference in diet of the two species. The herbivorous rabbit has a diet in which base, and particularly potassium, is in excess and may be stored in the tissues. The carnivorous rat, on the other hand, has a diet in which base is deficient and must be conserved. Rous' observations were made on the mouse. It would be of interest to know whether the active tissues of the herbivora are as acid as those of the carnivora.

Attention must be called to the recent study of the changes in lactic acid, etc., in stimulated rat muscles by Cori and Cori (1). They found, on the average, more than twice as much lactic acid formed after 15 seconds of tetanus as is reported here. A possible explanation of the difference is that their muscles were excised after stimulation by a definitely supramaximal current, and killed in ice-cold trichloroacetic acid. On the average, 30 seconds elapsed between the end of stimulation and the killing of the muscle. It may be that their higher values are due to the phenomenon of "delayed lactic acid formation" on which so much emphasis has been placed by Embden, Lundsgaard, Meyerhof, and Hill (3).

What, then, is the source of the energy for contraction? As previously stated, it is some oxidative reaction. Presumably lactic acid is the substance oxidized. The table actually shows a somewhat lower lactic acid content in the muscles stimulated for 30 seconds than in those stimulated for 15 seconds. While the difference is slight, the total quantity of lactic acid that could be oxidized in such a brief period is less than 3 mgm. per cent, assuming the same order of blood flow through these muscles that has been determined for other species. The evidence is suggestive, and taken together with the previous work on rabbits gives a satisfactory working hypothesis.

SUMMARY AND CONCLUSIONS

1. The changes in phosphocreatine, hexosephosphate, and lactic acid in stimulated rat muscles have been studied.

2. The amount of phosphocreatine hydrolysis is found to be proportional to the amount of lactic acid formed, and to be sufficient to buffer the muscle against most of the lactic acid formed.

3. The hexosephosphate formed is shown to be quantitatively adequate to buffer against the remaining lactic acid; hence there is no reason to assume that the muscle fiber becomes more acid during activity.

4. When continuous tetanus is prolonged beyond 15 seconds, there is no further formation of lactic acid or hexosephosphate, nor any further hydrolysis of phosphocreatine; i.e., the oxygen supply has become adequate for all the energy demands of the muscle.

5. The data presented confirm the authors' theory that the normal energy-yielding reactions in muscle are oxidative.

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SOME QUANTITATIVE STUDIES OF THE LOCALIZATION OF URANIUM IN THE PRINCIPAL ORGANS OF RABBITS DURING THE COURSE OF URANIUM INTOXICATION BY USE OF THE MAGNETO-OPTIC METHOD

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The present investigation was undertaken for the purpose of studying quantitatively the localization of uranium in some of the organs, blood and urine of animals. In the small amount of work previously reported by Karsner (1) and Eital (2) mainly qualitative methods were used with the results recorded as positive or negative.

Allison and Murphy (3) have developed the method used in these studies. In this magneto-optic method, compounds are detected by light minima produced by the time lag of the Faraday effect. This method has been further developed and made quantitative, within certain limits, by Bishop and Dollins (4).

We know from MacNider's (5) (6) work that the most damaging effect of uranium nitrate is upon the kidney. Yet the question arises, does this uranium nitrate produce a damaging effect upon other organs and the capillary system of the animals? There is yet no definite evidence that the death of the animals from this poison is due exclusively to the effect of uranium on the kidneys. The possibility of determining quantitatively minute amounts of uranium, by a method combining speed with dependable results was most interesting to us. It was, therefore, decided to use it in a series of studies to determine quantitatively the localization of uranium in the blood, urine, kidney, and other organs of the body.

PROCEDURE. Eighteen rabbits were used in this experiment. We injected subcutaneously 0.5 mgm. of uranium nitrate per kilgram of body weight. The animals were sacrificed at definite intervals as shown in the table. The blood, urine, and organs to be tested for uranium were taken and ashed. With these well ashed, 1 cc. of concentrated hydrochloric acid was added to each dish to take up the uranium. After a few minutes 1 cc. of distilled water was added and the contents washed into small bottles, stoppered with paraffined corks, and were then ready for analysis.

TECHNIQUE IN USE OF THE MAGNETO-OPTIC METHOD. In using the magneto-optic apparatus for quantitative analysis, the sensitivity to any given element must be very carefully determined. (The sensitivity of this case is the smallest quantity of uranium which can be detected by the apparatus.) This was determined by placing known quantities of the uranium nitrate solution in a platinum dish, evaporating to dryness in the oven, ashing, and taking up the residue in hydrochloric acid and water in the same manner as the organs from an animal. The reading was then

TABLE 1

Results of analysis of blood, urine, liver, spleen, and kidney from rabbits by use of the magneto-optic method

(Results are expressed in per cent of uranium injected)

DURATION OF EXPERIMENT	U INJECTED	BLOOD	LIVER	SPLEEN	KIDNEY	URINE AND URINARY SYSTEM	TOTAL
hours	*mgm.*						
Blank	0.0	0.0	0.0	0.0	0.0	0.0	0.0
1	0.53	89.0	1.0	1.0	6.2	1.3	98.50
2	0.30	31.0	44.0	0.31	15.0	0.66	90.97
3	0.38	27.0	50.0	0.34	23.0	0.34	100.68
4	0.32	16.0	26.0	0.10	17.0	25.0	84.10
5	0.99	13.0	22.0	0.084	22.0	23.9	80.084
6	0.36	5.6	16.0	0.072	23.0	33.0	77.672
7	0.48	4.0	13.0	0.0014	17.0	45.0	79.0014
8	0.33	0.15	15.0	0.0017	18.0	47.0	80.1517
9	0.37	0.16	23.0	0.06	18.0	35.0	76.22
10	0.34	0.39	20.0	0.22	28.0	33.0	81.61
11	0.43	0.79	13.0	0.21	30.0	39.0	83.0
12	0.31	2.1	15.0	0.19	35.0	28.0	80.29
13	0.41	1.6	13.0	0.20	32.0	43.0	89.81
14	0.36	1.7	15.0	0.26	26.0	32.0	74.96
18	0.36	0.60	12.0	0.025	35.0	43.0	89.625
20	0.42	4.0	13.0	0.039	40.0	29.0	86.039
24	0.39	0.34	14.0	0.086	34.0	38.0	86.426

made, using the sample as prepared, according to the following procedure. First, the sample was diluted by successive steps until a concentration was reached in which the characteristic minimum of the most abundant isotope (7) of uraunim did not appear. Then the concentration of the solution was increased by small increments until the minimum just appeared. A concentration was always obtained in which the second most abundant isotope of uranium did not appear. From studies made on uranium compounds by Allison and Goslin (7) it is known that these two isotopes appear at only slightly different concentrations. This made for uniformity in the results. Second, the characteristic minimum of the most abundant isotope was also

caused to disappear by a series of dilutions. This concentration at disappearance served as a check on the first method of procedure.

The final result represents an average for at least these two trials. The average value for a number of such determinations became the sensitivity of the magneto-optic apparatus to this particular uranium solution, and was found to be 0.663×10^{-12} grams U/cc. With the sensitivity of the apparatus known, the amount of uranium in the various organs, blood, and urine could be determined. The concentration at which the minimum characteristic of the most abundant isotope of uranium made its appearance in the sample was a measure of the uranium in that sample.

Example. Rabbit 1 was injected with 2.25 cc. of $U_2(NO_3)_2 \cdot 6H_2O$, equivalent to 0.533×10^{-3} grams of uranium. At the dilution of 1.40×10^{-9} of the ashed blood unknown the minimum characteristic of the most abundant isotope just appeared and the condition as described for the final reading was obtained. Therefore the amount of uranium present in the blood equals

$$\frac{\text{constant}}{\text{final conc.}} = \frac{0.66 \times 10^{-12}}{1.40 \times 10^{-9}} = 0.47 \times 10^{-3} \text{ grams}$$

A total of 0.53×10^{-3} grams uranium had been injected. Therefore the percentage of uranium in the blood was

$$\frac{0.47 \times 10^{-3}}{0.53 \times 10^{-3}} \times 100 = 89 \text{ per cent.}$$

The two equations combined:

$$\text{per cent of uranium injected} = \frac{\text{Const.} \times 100}{\text{Amt. injected} \times \text{final conc.}}$$

Discussion. The total percentages found in each individual animal vary from a minimum of 74.26 per cent to a maximum of 100.68 per cent. It is realized that this is a considerable variation. However, one would expect to find the largest percentages of uranium in the animals sacrificed at the end of one, two, and three hours respectively, when most of it is still in the blood and very little of it has been absorbed by the various tissues of the body. Part of this can also be attributed to the varying degrees in which the animals responded to the uranium intoxication. Bishop and Dollins (4) state that with careful manipulation, the magneto-optic method can be made accurate within 10 per cent plus or minus. Taking this into consideration with the variation in the reaction to uranium of the various animals the results checked remarkably well, especially as the ashed samples were unknowns to the observer. In view of these facts, it is felt that the results, as obtained, are sufficiently accurate to be of value. No other method has been developed which can be used in determining quantitatively such minute quantities of metals in animal tissues.

Rabuteau (8) states that uranium is excreted in the bile. By the use of this method it was impossible to detect even traces of uranium in the bile and gall bladder at the end of twenty-four hours. This is in accord with some results as reported by Karsner (1). Yet he states that he found it in the bile from dogs in two instances. However, Karsner was unable to detect any uranium in the liver, spleen, blood, or urine of cats forty-eight hours after intraperitoneal injections, while we were able to find it in the afore-named organs at the end of twenty-four hours. In studying the results as shown in the table it is readily seen that large amounts of the uranium are retained by the liver as well as the kidney, even at the end of twenty-four hours. In view of this information one might think that the uranium would exert an appreciable toxic effect on the liver. Should this be the case, then the deaths resulting from uranium injections would not be due entirely to the effects of the metal on the kidney, but probably to the combined effects on both the liver and kidney.

The process of elimination of the uranium by the kidney is begun during the first hour. The quantity continues to increase for each hour with one exception through the first eight hours. From the ninth to the twenty-fourth hour it varied from 27 per cent to 43 per cent.

SUMMARY

1. The magneto-optic method has been used advantageously and successfully in the quantitative determination of uranium in the blood, urine, kidney, spleen, urinary system, and liver, and the results tabulated.

2. No uranium was to be found in the bile, or gall bladder.

3. No other tissue was examined.

4. An average of 20 per cent of the total uranium was found in the liver. The maximum was 50 per cent and the minimum was 1 per cent, and 23 per cent in the kidney, with a maximum of 39 per cent and a minimum of 6.23 per cent.

5. The uranium is excreted through the kidney during the first twenty-four hours and not through the bile.

6. None of the metal was found in the tissues examined from the animal which was not injected with the uranium solution.

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PROTEINS AS STIMULANTS FOR THE SECRETION OF PEPSIN

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Throughout the many studies which have been made on gastric secretion, little attention has been directed to the factors influencing the physiological secretion of pepsin. Despite Heidenhain's early establishment (1879) of the independence of peptic and acidic secretion, there has existed an apparent tacit assumption that a gastric secretagogue is *per se* a peptagogue. This has been particularly true where food has been the secretory stimulus. Further, the few studies which have considered pepsin separately, chiefly those of the Pavlov school, have used diets of uncertain composition such as raw meat, bread or milk (Chishin, 1894). Pavlov himself (1910) states that the secretion of pepsin is dependent on the amount and character of nitrogen in the diet. We have endeavored to check this statement using diets of known composition which were varied only with respect to the amount and kind of protein present and employing the sensitive method of Gilman and Cowgill (1930) for determination of the quantity of pepsin present.

EXPERIMENTAL PROCEDURE. *Animals and care.* Mongrel dogs which had undergone Pavlov's operation for the formation of an accessory stomach at least six weeks prior to the first observation were used. These animals were housed in individual metabolism cages where they were kept during the collection of juice as well as at other times. The surrounding conditions were kept as constant as possible during experiments, and every effort was made to avoid both disturbing external influences and the formation of interfering conditioned reflexes.

Gastric juice collections were made for one-half hour before and for nine hours after feeding.

Diets. Besides two basal rations formed from a commercial dog food,[2] nine purely artificial rations were prepared using seven proteins at levels

[1] These data form a part of the dissertation presented by Elizabeth R. B. Smith to the Faculty of the Graduate School, Yale University, May 1933, in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

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[2] "Bal-Ra," purchased from the Richmond Abattoir, Richmond, Virginia.

varying from twenty to forty per cent of the diet, by weight. These diets were made following the kilogram-unit plan commonly used in this laboratory and described by Cowgill (1923). Table 1 gives a typical diet in which casein, tested at two concentrations, was the protein used. The other proteins studied in these experiments were: meat residue, obtained from the Valentine Meat Juice Company, Richmond, Virginia, and fed at two concentrations, coagulated egg-white, lactalbumin, secured from the Casein Company of America, New York City, hemoglobin, technical obtained from the Eastman Kodak Company, Rochester, New York, mixed serum proteins, prepared in the laboratory, and edestin, secured from the Pfanstiehl Chemical Company, Waukegan, Illinois.

METHODS. Free and total acidities were titrated directly with N/50 NaOH to the endpoints of Töpfer's reagent (salmon pink) and of phenolphthalein respectively; these determinations were made immediately

TABLE 1

Composition of the casein diet used in "Experiment II"

SUBSTANCE	GRAMS	CALORIES	PER CENT
Commercial casein	7.60	29.8	41.0
Sucrose	9.05	36.2	48.8
Butter	1.30	11.7	7.0
Salt mixture*	0.20		1.1
Bone ash	0.40		2.1
Total	18.55	77.7	100.0

1 kilogram-unit 18.6 grams; 1 gram 4.19 calories.

* Karr-Cowgill salt mixture (Cowgill, 1923a).

after the collection of juice, and total chlorides were then measured by the Van Slyke procedure (Van Slyke, 1923) on the same samples used for the acid titrations. Peptic activities were estimated in each sample, using the Gilman and Cowgill (1930) development of the Gates (1927, 1930) method. Total pepsin values were calculated as the product of the units per cubic centimeter and the volume in cubic centimeters, these calculations were not made if the volume of the sample was less than 1 cc. Units are expressed on a scale where the activity of crystalline pepsin Northrop[3] in a concentration of 1 gram per liter is defined as 100.

RESULTS. Wide variations were found with the different diets as to the rate of secretion and the total volume of juice secreted in nine hours. Consequently, there were also great variations in the free and total acidities

[3] We are indebted to Dr. John H. Northrop of the Rockefeller Institute for Medical Research, Princeton, New Jersey, who was kind enough to furnish us with a solution of his crystalline pepsin.

of the various samples. The total chlorides, on the contrary, maintained a remarkably steady value as did the total pepsin contents per hour. Pepsin concentrations, therefore, were almost inversely proportional to the volume secreted as was found by Kudo (1909). A summary of the data obtained is presented in table 2.

Of the various diets examined, the commercial dog food showed the strongest secretagogic properties. As a stock ration this material was diluted with an equal weight of sucrose. Fed in that form, it excited a nine hour total secretion of from 30 to 110 cc., varying with the dog. Six animals were used in this test. The totals just stated, and those yielded by all the other diets, are the average figures for at least three days of collection during which the daily total for any one animal did not diverge

TABLE 2

Gastric juice data obtained from one dog (X) throughout the entire series of diets tested and showing which factors tend to be constant and which variable

DIET	TOTAL VOLUME SECRETED IN 9 HOURS	MAXIMUM FLOW		TOTAL CHLORINE AVERAGE	TOTAL PEPSIN AVERAGE
		Appeared during	Volume of sample		
	cc.	*hour*	*cc.*	*m.-equiv.*	*units*
Basal	110	5	18.0	158.5	240
"Bal-Ra"	139	4	23.3	160.4	241
Casein III	62	7	9.2	158.6	249
Experiment II	47	7	7.6	156.0	241
Meat residue I	47	7	8.0	158.4	241
Meat residue II	109	4 or 5	15.0	157.0	243
Egg white	105	5	16.0	158.0	245
Lactalbumin	29	5	4.5	158.5	240
Hemoglobin	73	6	11.3	158.5	245
Serum protein	73	6	11.1	158.7	243
Edestin	25	6	4.1	156.5	238

more than 3 per cent from the average figure. For example, dog X. gave totals of 110, 110.5 and 108.3 cc. for three separate experiments carried out on successive days.

Usually juice began to flow within ten minutes after the food was offered. The hourly flow increased until the fourth or fifth hour after feeding when the maximum was attained, then decreased less rapidly, being considerable even at the end of the observation period. The maximum here coincided with the time of the intestinal phase as observed by Ivy, Lim and McCarthy (1925). The free acid present was generally proportional to the rate of flow, the maxima for these two variables occurring simultaneously.

The pepsin concentration of the sample collected during the first hour was extremely high, probably as a result of the "flushing" effect produced

by the outflow of juice. Beginning with the second hour, however, this concentration fell to a much lower figure, the minimum pepsin concentration coinciding with the maximum rate of flow of gastric juice. The product of the volume secreted and the pepsin concentration in units per cubic centimeter was nearly a constant figure, characteristic for each animal but not greatly different throughout the entire group. The data in table 3 illustrate this clearly.

When the commercial dog food was given without being mixed with sucrose, thus doubling the protein concentration (on the basis of total nitrogen the change was from 16 per cent to 30 per cent), the volumes secreted by each animal over the nine hours were much higher than those obtained when the usual basal regimen was fed. These totals were from

TABLE 3

Average total pepsin contents per hour

The values given below are in terms of a scale where the activity of crystalline pepsin (Northrop) in a concentration of one gram per liter is given a value of 100.

DIET	DOG							
	"X"	"Y"	"M"	"Z"	"D"	"H"	"T"	"C"
Basal	240	315	211	268	271	263	—	—
"Bal-Ra"	241	315	—	—	—	—	—	—
Casein III	249	312	212	264	272	263	298	266
Experiment II	241	316	210	263	273	—	—	—
Meat residue I	241	314	212	263	273	—	—	—
Meat residue II	243	312	211	263	—	—	—	—
Egg white	245	315	212	265	—	—	—	—
Lactalbumin	240	311	207	—	—	—	—	—
Hemoglobin	245	314	212	—	—	—	—	—
Serum protein	243	314	212	—	—	—	—	—
Edestin	238	314	207	—	—	—	—	—

one and one-half to twice as great as those obtained with the sugar-diluted ration, suggesting that protein concentration may have an effect on volume of secretion of gastric juice. The free and total acid values were also correspondingly increased, and the hour of maximum flow was slightly earlier than that for the sugar-diluted material. Total chloride concentrations were characteristically constant as were the calculated total pepsin contents, since the pepsin concentrations were greatly diminished below those obtained with the basal ration.

Casein. One of the two casein diets studied was a ration long used in this laboratory and denominated "Casein III" (Cowgill, 1923a); it contains about 40 per cent of commercial casein and 25 per cent of fat and is known to be low in content of the vitamin B complex. With this diet, however, the feeding periods were sufficiently short (6 to 7 days) so that this defici-

ency did not affect the results, as the animals exhibited no anorexia whatsoever. The effects of this regimen were studied with eight dogs.

The volumes of juice obtained on feeding Casein III were exceedingly low both for hourly samples and for the nine hour totals; the latter ranged from 15 to 60 cc. The maxima observed were not much greater than the average rate of flow and occurred later in the secretory period, at the seventh hour. The acid values were correspondingly less than those found with the two forms of commercial dog food, but the total chloride concentrations and the hourly total pepsin outputs were as constant as before. Pepsin concentrations were naturally somewhat higher with Casein III, with not so marked a falling off at the second hour.

It was thought that the low volumes and delayed occurrence of the maximum rate of secretion in the case of the tests with Casein III might be due to its comparatively high fat content (25 per cent); therefore, a second casein diet was prepared (expt. II) in which the proportion of protein was kept practically constant while the fat was decreased to about one-third of its former value. Tests of this new diet in which there was a diminution of fat content did not result in an increase in the volume of secretion; indeed, the nine hour totals were slightly less than those observed with Casein III itself, whereas the hour of the maximum flow was the same as before, suggesting that the characteristic observations made with the Casein III ration are to be attributed to the protein and not to the fat in the diet. With experiment II, the chloride concentrations and hourly pepsin values were constant as before.

Muscle proteins. Meat residue was used as a source of protein in two experimental diets at levels of 30 per cent and 45 per cent respectively. In the first case, the values obtained for all the factors observed were identical with those found for experiment II. When the proportion of the meat residue was raised from 30 to 45 per cent (protein concentration estimated from total nitrogen—from 20 to 30 per cent), the volume of juice secreted in nine hours increased to a value near that obtained on feeding the basal diet (stock dog food and sucrose); the hour of the maximum rate of flow was slightly earlier but the values for all of the other variables approach closely those obtained with the basal ration, in which the protein content, estimated from the total nitrogen, is scarcely half that of the 45 per cent meat residue regimen (see tables 2 and 3). This finding would suggest that the level of protein is not the only factor determining the course of secretion since different proteins at different levels produce identical effects.

Egg white. When coagulated egg white was used as the source of protein in a diet similar in structure to those of casein and meat residue, the response again closely resembled that obtained with the basal ration; here even the hour of maximum flow was also the same. This further finding

confirms the conclusion reached above as the coagulated egg white furnished 20 per cent of the diet.

Lactalbumin. This protein, when fed so as to constitute 20 per cent of the diet, produced results quite different from those just described. Here the volumes secreted, and, therewith, the acid values, were extremely low, being slightly less than those of experiment II (the casein low fat diet). These low volumes resulting from both casein and lactalbumin suggest that Chishin's (1894) results from milk feeding were not solely due to the liquid character and fat content of that food, as he believed. The total chlorides and total pepsin values of the juice secreted in response to the lactalbumin ration were comparatively constant, as in the tests with the other regimens. (See tables 2 and 3.)

Hemoglobin. Hemoglobin as a source of protein and constituting 20 per cent of the diet excited a flow of gastric juice, the nine hour totals of which were intermediate in value between those obtained with the basal diet and those observed with Casein III; the hour of maximum flow, however, corresponded to that of the latter despite the lower fat content of the hemoglobin ration. The pepsin content and total chloride concentrations were constant for each hourly sample as before.

Serum proteins. Mixed serum proteins, prepared from beef blood by heat coagulation, were also fed as approximately 20 per cent of the diet and gave results practically identical with those obtained from hemoglobin.

Edestin. The only vegetable protein available for our studies was edestin which was incorporated in the diet at a 20 per cent level. The total volumes of gastric juice secreted in response to this ration were even lower than those obtained with lactalbumin, being less than 30 cc. for the dog secreting the largest volume; this same animal secreted approximately 100 cc. in response to the basal diet and 60 cc. when Casein III was fed. The low volume with edestin is especially interesting in view of the fact that commercial dog food used as our stock ration contains large quantities of whole grains. The acid titration values with edestin were small as would be expected from the low volumes, but the total chlorides and total pepsin values were constant at their normal figures.

Discussion. The variations observed in the flow of gastric juice in response to the different diets were not due to a difference in volume or consistency of the rations since the weight of a kilogram-unit did not vary widely in the group of diets and because each portion was mixed with 100 cc. of distilled water just prior to feeding. In this way the consistency of the diets was kept as constant as possible.

From these several tests on diets containing different amounts of protein, several conclusions can be drawn, but two are especially noteworthy: the *constancy of the total chloride concentration* of the gastric juice as has been previously observed by Gamble and McIver (1928), MacLean,

Griffiths and Williams (1928) and others, and the *relative constancy of the total pepsin secretion per hour after the washing out which occurs in the first rush of secretion.* This pepsin constancy has been observed by Gilman and Cowgill (1931) in connection with stimulation by histamine but has not been recorded hitherto under physiological conditions of secretion. It should be repeated that the method of calculation of total enzyme present here consisted in the sample multiplication of the units per cubic centimeter by the volume of the secretion. For a like calculation with the Metts tube method of pepsin estimation the Pavlov school has used the Schütz-Borissov formula (Babkin, 1928), so that it is difficult to compare their pepsin results with those of the present study. Moreover, Patterson and Adler (1932) and others have questioned the validity of the use of the square with the data obtained by the Metts tube technique and have suggested the cube and other functions as more accurate representations of the facts.

The Gilman and Cowgill (1930) pepsin titration as used here has been based on the same standard pepsin throughout; consequently, although our results may not be directly comparable with those obtained in other laboratories, they can certainly be validly compared among themselves; therefore, when they reveal a constancy, this may be regarded as real and not apparent.

Pavlov (1910) stated that the quantity of enzyme produced is proportional to the amount and character of the nitrogen present in the diet, a statement which, when viewed critically, says little more than this: the amount of enzyme to be expected in response to a diet, 30 per cent of which is a given protein, for example, should be twice that obtained when only 15 per cent of the same protein is fed. In the present investigation, this was found to hold true for the volume of juice secreted, as was observed by Chishin (1894), but not for the *pepsin content, which remained constant regardless of the amount and kind of protein nitrogen given.* Some of these disagreements are doubtless to be attributed to the differences in methods of pepsin estimation and of computation of total enzyme content.

Chishin (1894) used bread as his source of vegetable protein and found a greater concentration of pepsin secreted in response to it than to animal protein, represented by raw meat. We did not find this to be true when pure edestin was the source of vegetable protein, if the difference in volume secreted is taken into consideration. The volumes found by the Pavlov school with bread feedings were also lower than those obtained with meat or with milk after the first hour. It would seem, therefore, that the results of Chishin and those obtained by us are in accord with respect to vegetable protein.

With raw meat, Chishin found the first hour sample, which was also the greatest in volume, to contain the highest concentration of enzyme. This

is in accord with the "flushing" effect on the glands postulated by Gilman and Cowgill (1931) with histamine and confirmed with food in the present study.

According to the Pavlov school, acid titrations are roughly proportional to the rate of flow of gastric juice. We join many other investigators in confirming this observation. Most of the variations found by us can be accounted for on the basis of the amount of mucus present.

It has long been the general view, based on experiments by Piontkowski (1906) on olive oil, that fats inhibit the secretion of gastric juice until they have been hydrolyzed to form soaps. Gordejew (1906) found that the addition of fats to other foods temporarily inhibited their action on the gastric glands. In the present studies, evidence has been adduced to show that this action is not always as great as has been previously supposed: decreasing the fat content of a casein diet from 25 per cent to 8 per cent had little effect on the volume of juice or the duration of secretion of the stomach. Fat when fed alone, or so as to form a large part of the diet, may inhibit the secretion of gastric juice, but small amounts of it incorporated in the diet appear to have little effect in this respect.

SUMMARY—CONCLUSIONS

Two basal rations formed from a commercial dog food, and nine artificial diets in which the protein was made the variable in quality and quantity, were fed to Pavlov gastric pouch dogs, and the secretion of gastric juice followed over a period of nine hours. The proteins tested were casein, meat residue, coagulated egg white, lactalbumin, hemoglobin, mixed serum proteins and edestin. In addition to observations of the rate of flow, the juice was examined for free and combined acid, total chloride and pepsin.

Although there were marked variations in the responses with respect to the rate of secretion, the total amount of juice secreted in nine hours, and the free and total acidities, the values for total chlorides and for total pepsin content, on the other hand, were remarkably constant. Pavlov's statement, that the quantity of enzyme produced is proportional to the amount and character of the nitrogen present in the diet, is not confirmed by these observations made with different proteins as the sources of nitrogen.

Casein, lactalbumin and edestin were the poorest secretagogues; hemoglobin and mixed serum proteins were slightly more effective, coagulated egg white proteins and muscle protein free from extractives and fed so as to constitute 45 per cent of the diet, were more effective, approaching in potency the basal stock diet which was a complex mixture. The conclusion is drawn that the secretion of pepsin under these physiological conditions is remarkably constant.

Each diet was tested on from three to eight pouch dogs.

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